STUDIES ON THE BIOLOGY OF SOIL-DWELLING NEMATODES OF TUSSOCK GRASSLAND

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Corrected copy.

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1. INTRODUCTION

Tussock grassland is the indigenous grassland of New Zealand. It covers one-fifth of the land area and is related to the "steppes" of the northern hemisphere (Zotov, 1939; Allan, 1946). Two types of grassland exist; tall tussock grassland which is dominated by plants of Chionochloa species, and low tussock grassland which is dominated by bunch grasses of Festuca and Poa species (Barker, 1953). The low tussock grasslands occur mainly in the South Island, covering almost five million hectares (Cockayne and Levy, 1956). During the early period of European settlement and agricultural development, montane tussock grasslands represented one of the few natural resources that maintained agricultural production without modification (Cockayne, 1915); but continued mismanagement by burning and overgrazing led to changes in species composition, and to deterioration and erosion in many areas (Cockayne A., 1910, 1915; Cockayne, 1919a, 1919b; Zotov, 1938; Cumberland, 1945; Raeside and Gibbs, 1945). With a steady decline in the relative value of production from the high country grasslands, and a growing realization of the importance of soil and water conservation, interest in the ecology of tussock grasslands intensified through the 1950's, culminating in the formation of the Tussock Grasslands and Mountain Lands Institute in 1960.

The ecological problems of tussock grassland management are well documented (see Hercus, 1956; Drummond and Leatham, 1959; McCaskill, 1963; O'Connor, 1966; Tussock Grasslands and Mountain Lands Institute Review, 1961-1970). However apart from entomological considerations (Dick, 1940; Kelsey, 1957), studies on the soil fauna and micro-flora were

neglected until Thornton (1958a) introduced a co-ordinated effort by a group of workers on the biology of tussock grasslands soils. The programme included investigations on distribution and population dynamics of fungi (Thornton, 1958b), yeasts (di Menna, 1958), bacteria (Stout, 1958a; Ross, 1958), protozoa (Stout, 1958b), streptomycetes (Vernon, 1958), algae (Flint, 1958) and earthworms (Lee, 1958), with respect to three tussock grassland soils. This was followed by a parallel study of fungi (Thornton, 1960b), yeasts (Di Menna, 1960), bacteria (Stout, 1960a; Ross, 1960) and protozoa (Stout, 1960b) in cultivated and uncultivated tussock grassland soils (Thornton, 1960a). Subsequent work on the bacterial flora was carried out by Robinson (1962) and Robinson and MacDonald (1964). More recently, attention has been drawn to the problems involved in the establishment of legumes and nodule-forming bacteria in tussock soils (e.g. Blair, 1967; Adams and Lowther, 1970).

Knowledge of several groups of organisms in high country soils is lacking (Hayward, 1967), nematodes included. Nematodes constitute an important part of the soil fauna .... "being more numerous than any other animal of comparable size. Obviously they must be considered in any comprehensive study of soil biology, " (Christie, 1959). The present project was initiated with the support of the Miss E. L. Hellaby Indigenous Grasslands Research Trust, to investigate the biology of nematodes in a tussock grassland soil.

From an agriculturist's viewpoint, ecological research is essential to the understanding of nematodes as factors in the biology of the soil, and to the interpretation of plant-nematode interactions in the aetiology of plant disease. Despite the significance of such studies and the increasing interest in soil biology, there are many gaps in current knowledge. As might be expected, the economically important plant parasitic nematodes have received most attention

(e.g. Dropkin, 1955; Christie, 1959; Jones, 1959, 1965; Seinhorst, 1961; Thorne, 1961; Oostenbrink, 1966; Jenkins and Taylor, 1967; Paramonov, 1968); for more general reviews see Nielsen, (1949, 1967), Winslow (1960), and Wallace (1963). Although there is a considerable volume of literature on various aspects of nematode ecology there have been few attempts to interpret pattern and process in a nematode population as a whole. Too often, ecological discussion is restricted to consideration of physico-chemical factors on populations without due regard to the ecology of the species involved and their interactions with the various components of the soil fauna and flora.

In view of the lack of information on nematodes in tussock grassland soils in New Zealand, and the lack of understanding of many aspects of nematode ecology in general, studies were undertaken , with the following objectives:

i) to consider the interrelationships between nematodes and the soil fauna and flora under laboratory conditions, and to study the biology of species representing a range of ecological niches in the soil;

ii) to investigate nematodes in the field situation by considering the distribution and annual variation of the populations with regard to changes in biotic and abiotic factors of the environment and the biology of the species involved;

iii) to investigate the significance of plant parasitic nematodes in causing damage to tussock grassland plants.

2. GENERAL

2.1 INTRODUCTION

"Before a community can be described and its structure studied, much technical and taxonomic work has to be done. Technical work in the sense that sampling procedure has to be elaborated and the appropriate extraction techniques chosen" (van der Drift, 1967).

During the initial stages of the study, sampling and extraction procedures were evaluated and standardised. In the course of this work the predominant nematode species of the fauna were characterised and an indication of relative abundance obtained.

2.2 STUDY AREA

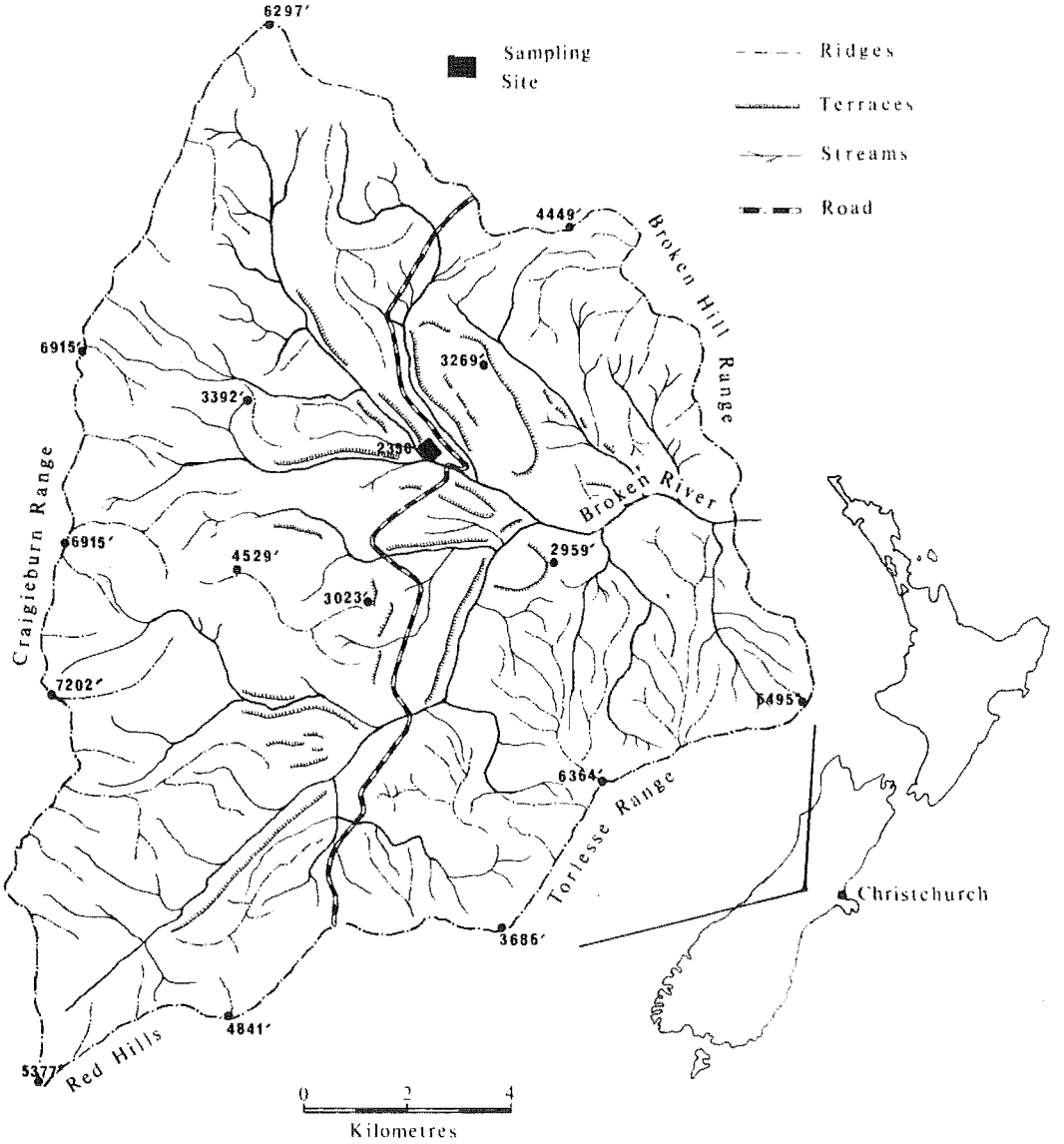
Preliminary samples were drawn from a range of sites up to 10 kilometres apart in the Waimakariri Catchment of the Southern Alps of New Zealand. As a result of the variability expressed by the nematode fauna, both between areas and between samples within areas, further sampling was restricted to an area of about eight hectares at Broken River in the Castle Hill basin (N.Z.M.S. 10A, Sheet 25).

The site was chosen because it included the Department of Agriculture experimental station for which agronomical and meteorological records were available, and because previous work had been carried out on the microbiology of the soil (Thornton, 1960a, 1960b; Stout, 1960a, 1960b; di Menna, 1960; Ross, 1960).

2.2.A Aspect

The study area (Figures 1 and 2) is located on ploughable terrace country at an altitude of about 720 metres. It is exposed to north-west and southerly winds and winter snows, and has an annual rainfall of about 114 cm (Dingwall,

FIGURE 1. Location of sampling site.


6297′
Sampling Site
Ridges
Terraces
Streams
Road
4449′
Broken Hill Range
6915′
3269′
3392′
2330′
Broken River
Craigieburn Range
6915′
4529′
2959′
3023′
7202′
5495′
6364′
Torlesse Range
Christchurch
3686′
4841′
5377′
Red Hills
0
2
4
Kilometres

1956). Within the confines of the terrace, two sub-plots, each covering approximately 2,000 square metres were sampled. One sub-plot was situated on an area of agriculturally improved tussock grassland, and the other about 200 metres away on unimproved tussock grassland.

2.2.B History

The improved area had been overdrilled in 1954 with a permanent pasture seed mixture (Appendix I), and top-dressed annually until 1964 (Appendix II), with the additional application of 3,763Kg of super-phosphate per hectare during the subsequent period (Dingwall, pers. comm., 1970). The unimproved area has been modified by grazing, by burning and by the invasion of some exotic species, but has not been cultivated, oversown, or fertilised. Both areas have been subject to periodic grazing by cattle and sheep.

2.2.C Vegetation

Fescue tussocks (Festuca novae-zelandiae C.) are the physiognomic dominants on both sites. The exotic grasses, sweet vernal (Anthoxanthum odoratum L.) and brown top (Agrostis tenuis Sibth.) are dominant in the inter-tussock zone.

2.2.C.I Improved tussock grassland

Individual tussocks are larger than those growing in the unimproved soil. The inter-tussock zone is well covered with a close sward of pasture species which include, brown top; sweet vernal; white clover (Trifolium repens L.); Montgomery red clover (Trifolium pratense L.); suckling clover (Trifolium dubium Sibth.); perennial ryegrass (Lolium perrene L.); crested dogstail (Cynosurus cristatus L.); Yorkshire fog (Holcus lanatus L.); timothy (Phleum pratense

FIGURE 2. Sampling area.

L.); goosegrass (Bromus mollis L.); mouse-eared chickweed (Cerastium holosteoides Fr.); catsear (Hypochaeris radicata L.), and patches of moss (e.g. Tortula princeps De Not. and Bryum species) are also common.

2.2.C.II Unimproved tussock grassland

Common inter-tussock species include , brown top; sweet vernal; scabweed (Raoulia subsericea Hook.); crane's-bill (Geranium microphyllum Hook.); bluebell (Wahlenbergia albomarginata Hook.); Pimelea oreophila Burrows; Discaria toumatou (Raoul); Coprosma spp., and the moss Rhacomitrium lannginosum (Hedw.) Brid.

2.2.D Soil type

The soil at Broken River is included in the yellow-brown earth group (N.Z. Soil Bureau 1968). It is a Cass fine sandy loam, moderately weathered and moderately to strongly leached, formed on low tussock grassland on greywacke alluvium (Thornton, 1960).

Description:

10-15 cm., dark brown fine sandy loam; friable, weakly developed medium and fine nutty and fine crumb structure;

30 cm., yellowish brown sandy loam, friable; weakly developed block structure;

on pale yellowish grey silt over deep gravel.

2.2.E Soil analyses

Results of analyses of soil samples carried out at the Invermay Agricultural Research Centre, Mosgiel, are presented in Table 1. A low phosphate status is indicated, and K would be deficient in the inter-tussock sample from improved grassland (Grigg, pers. comm., 1971).

TABLE 1: Analyses of soil samples from 0-10cm depth.

Test	Site			
	Improved grassland		Unimproved grassland	
	Tussock	Inter-tussock	Tussock	Inter-tussock
pH	6.0	6.2	5.7	5.7
N%	0.28	0.36	0.25	0.24
C%	4.2	5.3	4.1	3.5
C:N	15	15	16	15
*Ca	5 (3.1)	8 (5)	3 (2.2)	3 (2.2)
*K	7 (0.36)	3 (0.15)	12 (0.61)	11 (0.56)
*P (Truog)	3 (1.2)	3 (1.2)	3 (1.2)	1.(0.4)
*P (Bray)	19 (1.9)	11 (1.1)	10 (1.0)	9 (0.9)

*Dept. Agric., 'quick tests'.
Ca and K: Extracted by neutral 1 molar ammonium acetate, and measured from the filtrate by flame emission. Ca expressed as p.p. 40,000 of extract; K expressed as p.p. 250,000 of extract. Approximate m.e.% are given in brackets.
P (Truog): Extracted with Truog reagent (0.002 N H_2SO_4 containing 3g $(NH_4)_2SO_4$/litre) and P determined by the ascorbic acid method. Results expressed as p.p. 50 x 10^6 of extract. Approximate mg/100g given in brackets.
P (Bray): Extracted with 0.03 N NH_4F plus 0.025N HC1, and P determined by the ascorbic acid method. Results expressed as p.p. 10 x 10^6 of extract. Approximate mg% 100 g given in brackets.

2.3 ASSESSMENT OF THE NEMATODE FAUNA

2.3.A Qualitative assessment

To obtain large numbers of animals three methods were used:

(i) Baermann funnel extractions (Cairns, 1960)

(ii) Cornmeal agar "soil plates". Petri dishes containing about 15 ml of Difco cornmeal agar were seeded with 1-2g of soil. The nematode fauna which developed over three months was examined at regular intervals. Populations were maintained by periodically replenishing the cultures with a film of 0.8% water agar poured at 40-45°C.

(iii) Pea-agar "buried plates". The bottom section of a disposable Petri dish was perforated with three holes of about 3mm diameter. The base was placed within the inverted lid and poured with pea-agar (Appendix III). When the medium solidified the lid was placed in the normal position and the surplus medium removed from the underside of the base. To place the dishes in the soil, a block of turf was first removed to a depth of about 15cm. The pea-agar plates were positioned vertically, with the perforated base against the cut face and the uppermost lip about 2-3cm from the soil surface and the turf replaced. After four to six weeks most cultures supported large populations of nematodes, as well as populations of mites, collembola, millipedes, and other faunal groups.

2.3.B Quantitative assessment

2.3.B.I Selection of extraction method

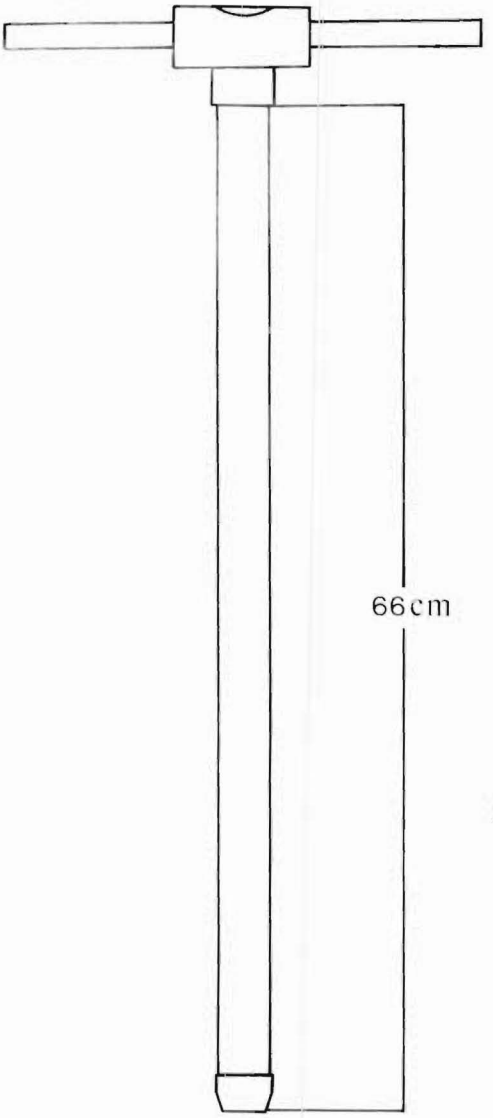
Cairns (1960) reviews the various methods used for the quantitative extraction of nematodes from the soil. In the present study the centrifugal-flotation method of Caveness and Jensen (1955) as modified by Miller (1957) was compared with Seinhorst's elutriation and differential sieving method (Seinhorst, 1956; 1962b;Goodey, 1963a). Seinhorst's method proved superior by up to 50% for total recovery of nematodes. In a similar comparison, Zuckerman, Khera and Pierce, (1964) reported higher yields of Hemicycliophora similis Thorne using centrifugal-flotation but found that recovery of nematodes of other genera was low.

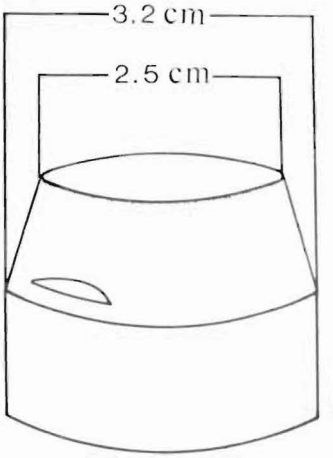
Seinhorst's method with some modifications (see below) was adopted for subsequent extractions.

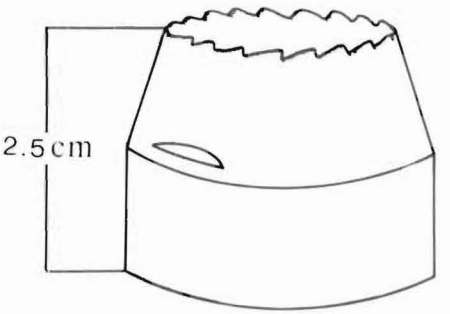
2.3.B.II Sample size

Andrassy (1962) investigated the effect of sample unit size on population estimates of soil nematoda. By increasing the size until the number of species and the number of individuals of characteristic species was relatively constant, he concluded that 1cm diameter sampling units are adequate for population density estimates. On the basis of Andrassy's (1962) discussion the cores of $5cm^2$ cross sectional area taken in the present study are considered to be representative. To minimize compaction during penetration of the soil and to facilitate clean removal of the cores, the cutting tip diameter of the soil corer was 4mm narrower than the internal diameter of the corer shaft. During the winter months the frozen surface layer of soil could not be penetrated with a standard knife-edge cutting tip. Under these conditions a saw-edge cutting tip of the same dimensions (Figure 3) was used.

FIGURE 3. Soil sampling equipment.
Diagram) Soil corer with knife-edged and saw-edged cutting tips.
Half-tone) Intact soil core.


66cm


3.2 cm
2.5 cm


2.5cm

Sample size was determined on a volume basis. Soil cores usually remained intact on removal (Figure 3) and could be cut to the appropriate length. However, during the summer drought samples tended to crumble. Under these conditions sample size was determined using a measuring cylinder. In preliminary trials large volumes of soil (up to 400ml) were processed following Seinhorst's recommendation for soils "rich in organic matter or mica," (Seinhorst, 1962b). The concentration of fine soil particles and organic matter proved troublesome in the sieving and counting processes and gave rise to inconsistent results. Increasing the elutriation time did not alleviate the problem, so samples of less than 250g (Seinhorst, 1962b) were considered as follows:

To test the effect of sample volume on the efficiency of extraction, seven cores, each taken to a depth of 7.5cm from an area of about 11cm^2 were thoroughly mixed. Then from this "homogenised" bulked sample subsamples of 37.5 and 150ml were drawn. These subsamples were processed according to Seinhorst (1962b). The results (Table 2) show that, overall, the smallest samples (37.5ml) yielded more nematodes per unit volume of soil. In larger samples the concentration of fine soil particles and organic matter proved troublesome in the sieving and counting processes which probably accounted for much of the disparity. A soil volume of approximately 37.5ml (30-35g) was used for subsequent extractions.

2.3.B. III Storage of samples

Soil cores were rolled in plastic bags and sealed with rubber bands in the field and transferred to a 4^{o}C storage room (Goodey, 1963a). Up to five hours elapsed between collection of samples and placement in cool storage.

TABLE 2: Numbers of nematodes extracted by elutriation of three different soil volumes.

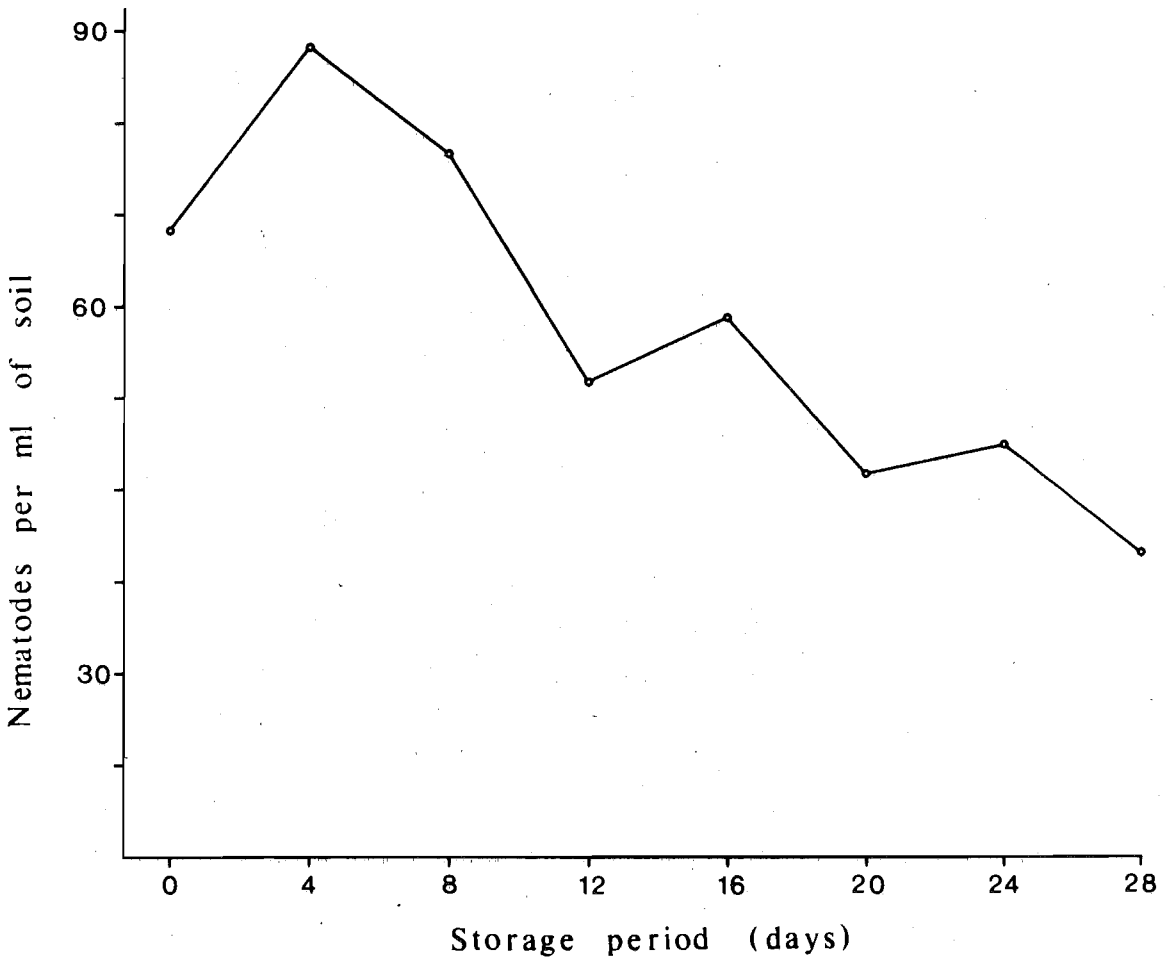
Soil Volume ml	Number of nematodes per ml of soil for three sample series			
	1	2	3	Mean
150.0	77.34	90.95	108.0	92.1
75.5	85.61	96.79	140.1	107.5
37.5	105.40	108.80	136.5	116.9

Counting and identification of animals were most efficient when "live samples" were used. When large numbers of cores had to be extracted a period of storage between collecting and processing samples was inevitable. The effect of storage time on the number of nematodes harvested per core thus became important and was tested. Eight cores were drawn from an area of about 11cm^2, sealed in plastic bags and placed in the 4^{o}C room. On the day of sampling and every fourth day thereafter, a core was taken at random and processed. The results in Figure 4 show a marked drop in nematode numbers over the duration of the experiment. While some of the variability can no doubt be attributed to between-core variability, the downward trend is definite. On the basis of these data the maximum "safe" period of storage under the test conditions was considered to be eight days.

2.3.B.IV Preparation of samples for elutriation

Soil cores contained matted plant roots and organic matter. To standardise the preparation of soil suspensions, mechanical maceration and dispersion techniques were employed. Each sample was immersed in 250ml of water and macerated in a Sunbeam Blender for 10 seconds at 3,000 r.p.m. and the resultant suspension was further dispersed by agitation for two minutes in a Vibromixer with a 54mm diameter perforated plate. The separation of plant material was achieved by pouring the soil suspension through a sieve (aperture size 1.5mm) over a collection tray (Figure 5 A and B). Any remaining soil was rinsed through the sieve using a jet of water. The plant material retained on the sieve was placed in a Baermann funnel and the nematodes collected after 24 hours were added to the total population obtained by elutriation. If species of typically endo-parasitic genera (e.g. Pratylenchus sp.) were observed, the material was extracted for a further 24 hours.

FIGURE 4. Relationship between time of storage of soil at 4°C and numbers of nematodes extracted.


90
60
30
Nematodes per ml of soil
0
4
8
12
16
20
24
28
Storage period (days)

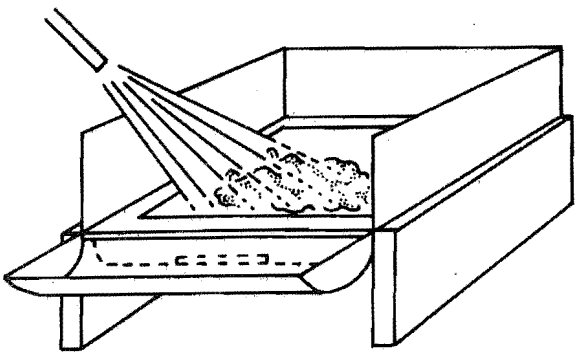
Counts taken for preliminary samples indicated that the nematodes harvested in the Baermann funnels rarely exceeded 3% of the total number extracted.

2.3.B.V Elutriation

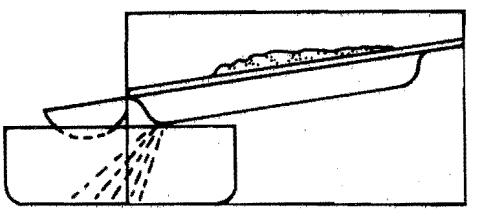
A two litre conical flask F (Figure 5C) containing the soil suspension was inverted over the top section of the Seinhorst apparatus, maintaining a continous water column. The inflow of water from the constant head tank H was controlled at 50ml per minute to standardise the flow rates in the elutriator tubes (see Seinhorst, 1962b)

A one litre glass bottle B, connected by a ground glass joint to section S_3 replaced the bottom section of Seinhorst's apparatus. A one-way tap T_1, with a 1cm diameter through-pipe controlled the flow of coarse material from S_3 to B. This obviated the need for an internal bung, and enabled a more efficient recovery of sediment from S_3 at the conclusion of the separation. The modification could be improved by increasing the diameter of the through-pipe, as occasional blockages in the present system had to be cleared with a wire push-rod manipulated from the top of the apparatus. After six minutes the tap T_1 was closed to prevent further sedimentation into the bottom of the vessel. Twenty minutes later, flask F was removed from the column and after a further 10 minutes the elutriation was terminated. All of the contents of the column above T_2 were collected in container C_2 and combined with suspensions from F and C_1. Seinhorst retained the suspension from S_2 and passed it through 100μ sieves (Goodey, 1963a). During the initial stages of the present study separate examination of the nematodes retained in the three sections of the elutriator revealed some small animals in S_2, (probably from the junction of S_1 and S_2). For this reason the suspension from S_2 was treated with suspensions from

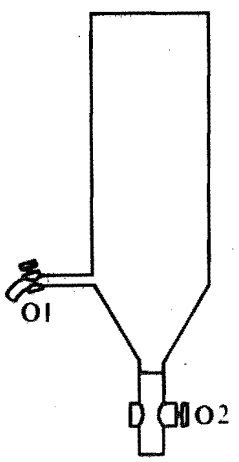
FIGURE 5. Apparatus for preparation and extraction of soil samples.
A - B) Sieve and collection tray for separation of plant material.
C) Modified Seinhorst elutriator.
D) Concentration funnel.



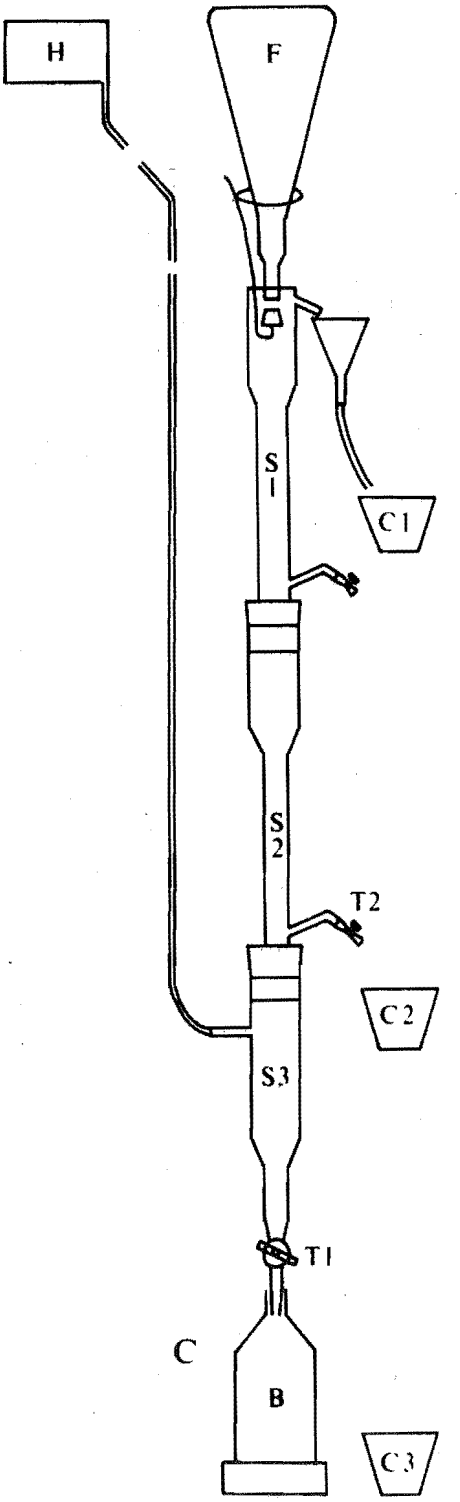
A



B



D



C

S_1 and F in the subsequent sieving processes. Container C_3 was filled from the section S_3 after removal of the bottom vessel B.

2.3.B.VI Sieving

The combined suspension from C_1 was passed three times through a bank of four 350 mesh sieves mounted at an angle of 10^{o}, and the residues were collected by rinsing both sides of the mesh. The contents of C_2 were poured twice through a 200 mesh sieve, collecting the residues from each. Further separation was achieved by gently pouring the collected suspension over a single thickness of paper "Snowtex" tissue placed on the surface of 68μ nylon gauze sieve (Yeates, 1968a) standing in a shallow dish. The dishes were filled until the water surface wetted the gauze. After 24 hours the sieves were rinsed into the dishes and the combined contents retained for further processing. Checks revealed that three to six percent more nematodes were harvested after 48 hours standing, but the increase was not considered to justify the additional time involved.

2.3.B.VII Concentration of samples

Before concentration the nematode suspension had a volume of about 300ml. Concentration by settling and suction (Seinhorst, 1956) proved time consuming and it was considered that small nematodes could be lost. Instead, the suspension was poured into a 450ml deep-sided funnel with a steeply narrowing bottom section (Figure 5D). After allowing two hours for settling, the water above the outlet O, was run off, and the concentrated suspension collected from O_2. The final volume of the sample after rinsing of the funnel was 40-50ml.

2.3.B.VIII Counting

Samples were poured into a boiling tube, adjusted to about 50ml and a 10ml aliquot drawn by pipette from the agitated suspension. The aliquot was transferred to a circular counting tray (Doncaster, 1962), and the total number of nematodes counted under a stereoscopic microscope at 50x magnification. Despite the careful sieving processes, fine soil particles and organic matter were retained in the final samples. Suspensions containing excessive detritus were diluted to 100ml and the population estimates based on counts of two 10ml aliquots.

2.3.B.IX Identification

The first 100 nematodes encountered in a 10ml aliquot of each sample were temporarily mounted in water for specific identification under a compound microscope. The composition of the nematode fauna was calculated from the percentages obtained.

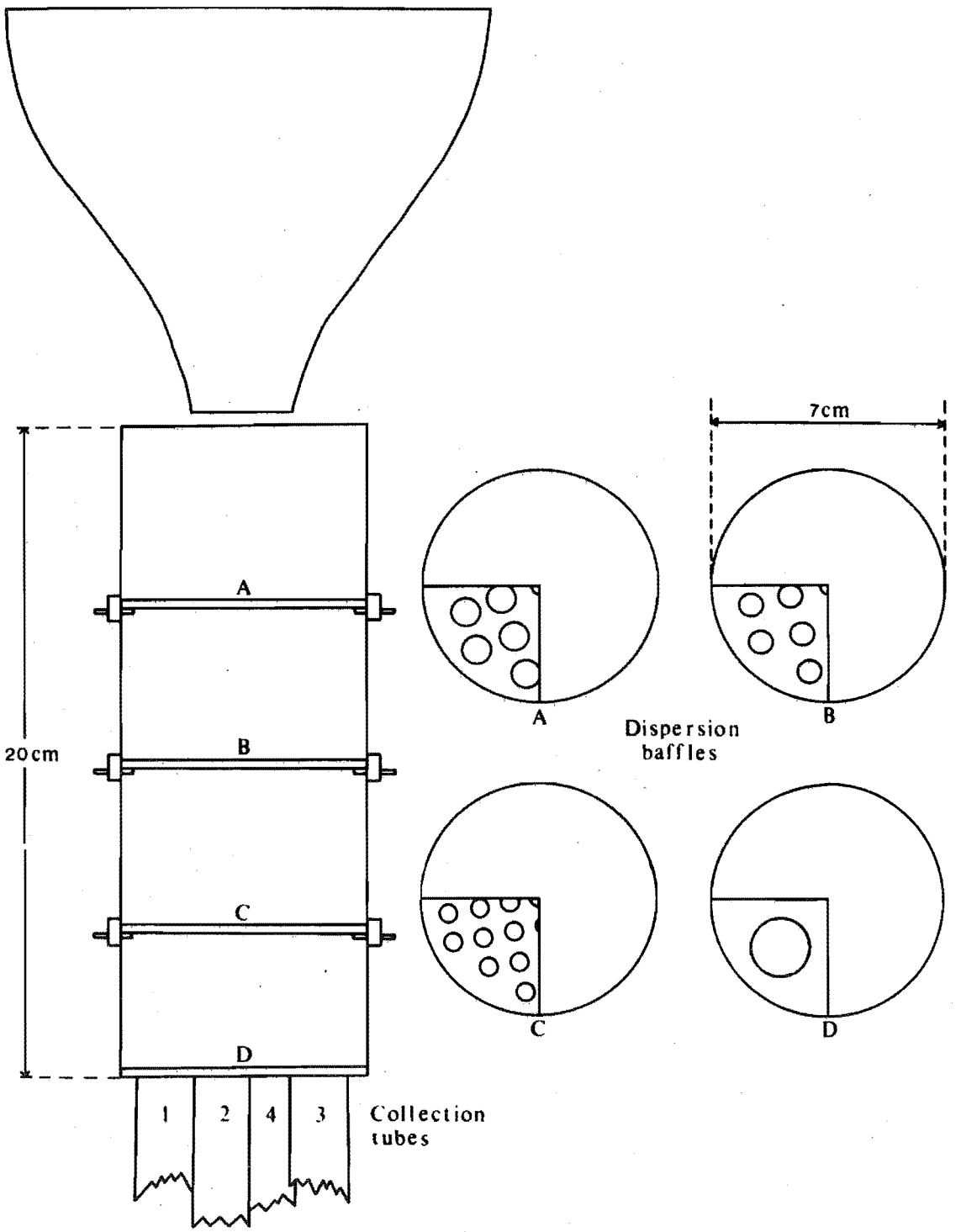
2.3.B.X Subsequent processing

Nematodes were heat-relaxed by immersing samples in a water-bath at 60°C, and fixed by addition of an equal volume of double strength TAF (Goodey, 1963a).

2.3.C Reliability of extraction

Four soil cores (each 37.5ml) were taken from the centre of a fescue tussock plant. The suspensions prepared from each core were combined and the slurry made up to about 4 litres. To obtain four random sub-samples the total volume was passed through a cylinder containing three perforated discs (Figure 6) which dispersed the stream of water and soil before it was collected in four outlet

FIGURE 6. Random sub-sampling apparatus.
A - C) Dispersion baffles.


7cm
20cm
A
B
C
D
1
2
4
3
Collection tubes
Dispersion baffles

tubes at the bottom of the column. Slight differences in final volumes were adjusted when necessary by pipetting suspension from one agitated sample to another. To test the reliability of the extraction procedure each sub-sample was treated as an independent sample in subsequent processing. Three estimates of the total population in each sub-sample were made from counts of nematodes in 20% of the total volume of the suspension (section 2.3.B.VIII). Analysis of variance between sub-samples (Table 3) shows that the extraction produced reproduceable results and that the sub-samples may be regarded as aliquots.

In counting nematodes in large numbers of extracts from field samples, one count per extract was made. To investigate the significance of the deviation from a 1:1:1:1 ratio in single counts for each aliquot, Chi-square analysis was used. Six series of aliquot counts were tested. The results (Table 4) show that approximately 12% of the counts differed significantly. The coefficient of variation for total population estimates based on the aliquot counts ranged from 4.4-14.9%, which is here regarded as satisfactory.

The possibility, that variation in estimates of nematode numbers between aliquots could produce significantly different populations over a number of samples, was investigated using Wilcoxon's signed rank test of difference score population symmetry about zero (Bradley, 1968). Pairs of counts were randomly drawn from each of the six "populations" of counts per 10ml of suspension. One pair from each count was randomly assigned to arbitrary populations A and B and each of the remaining two were randomly assigned to populations X and Y. No significant differences were detected, indicating identical effects of aliquots.

TABLE 3: Analysis of variance of estimates of total nematodes extracted from four independently processed sub-samples of a suspension of field soil.

Source of variation	df	SS	MS	F	F required		Result
					0.05	0.01	
Treatment	2	284344	142172	2.09	4.26	8.02	NS
Error	9	613174	68130				
Total	11	897518					

TABLE 4: Significance of the departure from a 1:1:1:1 distribution of nematode counts between aliquots.

	Aliquot number							
Series	Count 1	X^2	Count 2	X^2	Count 3	X^2	Count 4	X^2
1a	548	0.003	537	0.174	592	3.921	520	2.471
1b	429	6.855	451	2.626	569	13.910**	498	0.260
1c	551	11.170*	436	3.698	429	5.034	496	0.679
2	886	1.378	925	0.626	854	0.248	971	5.397
3	1051	14.170**	901	1.365	895	1.861	900	1.442
4	701	0.023	750	2.910	680	0.898	689	0.368

X^2 (3df)

0.05 = 7.815*

0.01 = 11.34**

1a - 1c: Replicated counts for one series of aliquots

2.3.D Efficiency of extraction

The relative efficiency of extraction methods for nematodes has been shown to vary (Whitehead and Hemming, 1965; Oostenbrink, 1970). To investigate the efficieny of the modified Seinhorst technique, two soil samples were processed separately and their respective nematode populations estimated. Each nematode suspension was then combined with 37.5ml of oven dry soil and the nematodes re-extracted. In the first instance a recovery of 79.8% was obtained, and in the second, 85.9% of the nematodes were recovered.

Yeates (1967) noted a disparity of extraction efficiency between nematode species using the Seinhorst elutriation process. In an experiment to investigate the relative extraction efficiency for different species, a population of nematodes comprising animals from laboratory culture (with the exception of Paratylenchus projectus) was added to 37.5ml of oven dry soil and processed. The results (Table 5) show that the recoveries for different species were inconsistent. Further, the overall recovery of 68.77% was less than that previously obtained. Different species composition of the populations examined probably contributed to the difference, but possibly more important was the fact that the composite sample from nematode cultures contained a large proportion of juveniles for each species. Seinhorst (1962a), found that by repeated sieving an almost complete recovery of nematodes from a suspension can be obtained if the sieve-mesh is 0.1 of the nematode length and reasonable recovery if the sieve-meshes are 0.2 of the nematode length. A large proportion of small juveniles in samples would result in a less efficient recovery. In re-extracted field samples selection against small juveniles and adults of some small

species occurs in the initial processing, thereby causing a bias to greater extraction efficiency in the final analysis.

Most of the losses were considered to occur in the sieving stages of the process. Re-extraction of the sediment from bottle B (Figure 5C) in Baermann funnels rarely yielded more than a few nematodes.

TABLE 5: Inocula and recoveries made using the modified Seinhorst technique

Species	Inoculum	Recovered	%Recovery
Paratylenchus projectus	2000	1336	66.8
Aglenchus neozelandicus	210	190	90.5
Tylenchus rikus	250	179	85.2
Aphelenchoides bicaudatus	400	312	78.0
Mesorhabditis sp.	200	135	67.5
Acrobeloides sp.	450	283	62.9
Wilsonema otophorum	150	70	46.7
Monhystera sp.	110	72	64.4
Tylencholaimus sp.	80	70	87.5
Aporcelaimellus paraamylovorus	30	21	70.0

2.4. SPECIES COMPOSITION AND FREQUENCY OF OCCURRENCE

The 69 species listed in Table 6 are those which were isolated on more than one occasion using the methods described in the previous section. Estimates of relative frequency are based on the results of 50 samples extracted

TABLE 6: Composition of the nematode fauna at Broken River

TYLENCHIDA

** Aphelenchoides bicaudatus
Aphelenchoides sp.
** Aphelenchus avenae
Bursephalenchus sp.
Cricenomoides sp.
Deladenus durus
Ditylenchus spp. (2)
Helicotylenchus sp.
Nothotylenchus sp.
***Paratylenchus projectus
Seinura demani
***Aglenchus neozelandicus
* Cephalenchus tahus
***Tylenchus maiakus
** Tylenchus rikus
* Tylenchus sp.
Tylenchus spp. (2)
Tylenchorhynchus sp.
Pratylenchus penetrans

RHABDITIDA

***Acrobeloides sp.
Cephalobus sp.
Chiloplacus sp.
* Cervidellis sp.
Cervidellis sp.
** Eucephalobus sp.
* Mesorhabditis sp.
Mesorhabditis sp.
* Rhabditis sp.
Diploscapter sp.
Diploscapteridae (2)
Pelodera sp.
* Genus et sp. indent.

CHROMADORIDA

Chromodorida sp.

MONHYSTERIDA

* Monhystera sp.
Prismatolaimus sp.

ARAEOLAIMIDA

Anaplectus granulosis
** Plectus sp. (P. armatus type)
* Plectus sp. (P. parietinus type)
* Plectus sp. (P. inquirenda type)
Plectus sp.
Bastiana sp.
Rhabdolaimus sp.
** Wilsonema otophorum

DORYLAIMIDA

** Aporcelaimellus paraamylovorus
Brachonchulus sp.
Diphtherophera sp.
***Dorylaimellus sp.
Dorylaimus sp. (Long tailed)
** Aporcelaimus sp.
Aporcelaimus spp. (2)
Eudorylaimus sp.
Labronema sp.
Mononchus propapillatus
Mononchus sp.
Mylonchulus sp.
** Nygolaimus (Paravulvus) sp.
* Tylencholaimellus montanus
* Tylencholaimellus sp.
***Tylencholaimus sp.
Miscellaneous Dorylaimida (6spp.)

*** Isolated from 50% of samples
** Isolated from 35-50% of samples
* Isolated from 20-35% of samples

by elutriation during preliminary surveys and investigations of nematode distribution.

2.5 DISCUSSION

To validate comparisons between nematode samples over space and time, extraction procedure and ancillary techniques were standardised. Seinhorst's elutriation method, modified to suit the prevailing conditions, was used in the main separation. The results were reproduceable for samples of the same soil composition, and the efficiency achieved fell within the range reported by previous workers. Seinhorst (1956) reported the recovery of 85-90% of the nematodes in samples of 500g using his elutriation method. In a later paper (Seinhorst, 1962a) recoveries of "at least 95% of the population" were claimed for his modified apparatus. Zuckerman, Khera and Pierce (1964), obtained recoveries of "about 65%" for smaller nematodes and "about 50%" for larger nematodes in extractions of 200ml samples using the Seinhorst elutriator. For extractions of duplicate samples they reported a variation of $\pm$ 15%. Whitehead and Hemming (1965), extracted about 70% of the nematodes from 200ml samples using their "sedimentation" method. In a comparison with three other methods, they extracted slightly more nematodes using Seinhorst's technique than by the sedimentation method; all four gave consistent results. Yeates (1967), recorded the recovery of 80-97% of relatively large nematodes from 250g sand samples, but obtained low efficiences (5-11%) for Mesorhabditis sp. Oostenbrink (1970) compared extraction methods in detail and reported recoveries of 55%, 80% and 55% for soil samples of 100ml, 50% and 90% for samples of 500g and 82% for 50ml samples, for the elutriation process. Further, in experiments to test the

reproduceability of extraction results, he recorded variations of up to 1:5.1 between four different operators. The disparity was in the same order for other techniques.

Extraction efficiency varies between species in the population and with the size of the nematodes within species. The effects of inconsistent recoveries between species may be significant in estimates of total numbers per core where the specific composition of the samples is different. But in comparisons of means over a series of samples the extent of the error is a function of the number of samples taken and the heterogeneity of the nematode population, and can be minimized by increased replication. But since extraction efficiency may also vary with the proportion of small juveniles in samples, during periods of rapid population increase, actual numbers may be under-estimated, causing a bias against the determination of relative maxima in population estimates over time.

3. FEEDING RELATIONSHIPS

3.1 INTRODUCTION

Ecology, by definition, involves the consideration of the interrelationships between an organism and its environment. Dao (1970) listed the physical, chemical and biological factors which are important in nematode ecology as climatic influences, food influences, influences of other organisms, and intraspecific influences. Knowledge of the feeding relationships of many species of nematodes is limited, indeed the feeding habits of some groups are unknown. Such basic information is prerequisite to considerations of the biology of nematodes and to the elucidation of the role of nematodes in the biology of the soil in general. The present chapter describes experiments designed to investigate the feeding habits of the most common nematodes in the Broken River fauna and to identify techniques that would enable subsequent detailed studies on the biology of selected species.

3.2 GENERAL

3.2.A Preliminary observations

Observations were recorded from populations of nematodes which had become established on cornmeal agar soil plates or pea-agar soil plates (see Chapter 2, section 2.3.A.).

3.2.B Surface sterilisation

Surface sterilisation of nematodes was necessary to minimise the carry-over of unidentified inocula with the test animals. This was achieved by immersing the nematodes for 24 hours in 0.9% water agar containing 100 p.p.m.

methoxy ethyl mercury chloride (Baytan) after the method of Goodman and Chen (1967). About 50% of the animals migrated to the surface of the medium and were recovered by rinsing with sterile water.

3.2.C Establishment of sub-cultures

Sub-culturing on to a range of food types was attempted on several occasions for the most common nematodes. Inoculum loads varied between 5-50 animals per 9cm Petri plate, depending on the availability of inoculum.

3.3 NON-STYLET-BEARING SPECIES

Nielsen (1949) demonstrated that populations of many species of Rhabditida and Araeolaimida could be established on culture media supporting colonies of bacteria. More recently, aspects of the biology of several rhabditid nematodes have been studied in monoxenic culture (Chuang, 1962; Thomas, 1965; Sohlenius, 1968a, 1969; Yeates, 1970), and species of the Araeolaimida have been cultured on bacteria (Dougherty, 1960; Maggenti, 1961). Some Rhabditida are known to be predacious (Sohlenius, 1968b; Yeates, 1969a). but predation was not observed in preliminary observations on species from Broken River, and the nematodes concerned are not generally regarded as predators. Hence culture experiments were designed on the premise that bacteria are the primary food source for this group.

METHOD

Xenic bacterial cultures: Nematodes from cornmeal agar soil plates were rinsed in sterile water and transferred to Petri plates containing soil extract agar (Pramer, 1965). On several plates, populations of eelworms

became established on the mixed bacterial flora carried over with the nematodes.

Polyxenic cultures: Nematodes were placed on mixed isolates of bacteria consisting of Araeomonas sp., Bacillus sp., Psuedomonas spp., (2), and Flavobacterium sp., cultured on soil extract agar or one-half strength Difco nutrient agar.

Monoxenic cultures: Progeny of nematodes which established populations on a mixed bacterial diet were extracted, surface sterilised, and placed on mono-specific isolates of Bacillus cereus, Escherichia coli, or Pseudomonas fluorescens cultured on soil extract agar, one-half strength Difco nutrient agar, or asparagine mannitol agar (see Goodey, J.B., 1963a).

Cultures of B. cereus, E. coli and P. fluorescens were obtained from the Department of Microbiology and Genetics at Massey University.

RESULTS AND DISCUSSION

Of the 18 species of nematodes investigated, only Prismatolaimus sp., and Rhabdolaimus sp., could not be cultured on any of the substrates tested (Table 7), although a large population of the latter species developed on cornmeal agar soil plate. Sub-culturing of Araeolaimids was unsuccessful when dense colonies of bacteria covered the agar surface. Hence soil extract agar was the best medium for these species, and populations of bacteria were incubated until the initial flush of growth collapsed before nematodes were added. Nielsen (1949) suggested that rapid bacterial growth induced mortality of eggs and larvae due to oxygen deficiency. In this respect Araeolaimida were more susceptible than Rhabditida. Populations of species of Rhabditidae and Cephalobidae were

TABLE 7: Results of feeding trials with non-stylet bearing nematodes

Nematodes investigated			Culture conditions				
			Xenic	Polyxenic	Monoxenic		
Order	Family	Species			*B. cereus*	*E. coli*	*P. fluorescens*
Rhabditida	Cephalobidae	*Acrobeloides* sp.	1	1	1	1	1
		Chiloplacus sp.	1	1	NT	1	NT
		Cephalobus sp.	1	1	NT	1	NT
		Cervidellis sp.	1	1	NT	NT	NT
		Eucephalobus sp.	1	1	1	1	1
	Rhabditidae	*Mesorhabditis* sp. 1.	1	1	1	1	1
		Mesorhabditis sp. 2.	1	1	1	1	1
		Pelodera sp.	1	1	1	1	1
		Rhabditis sp.	1	1	1	1	1
Araeolaimida	Plectidae	*Anaplectus granulosis*	1	1	1	1	1
		Plectus sp. 1 (*P. armatus* type).	1	1	0	1	NT
		Plectus sp. 2 (*P.parietinus* type).	1	1	0	0	NT
		Plectus sp. 3 (*P. inquirandus* type).	1	1	NT	NT	NT
		Plectus sp. 4	1	1	NT	NT	NT
		Wisonema otophorum	1	1	0	1	NT
	Leptolaimidae	*Rhabdolaimus* sp.	0	0	NT	NT	NT
Monhysterida	Monhysteridae	*Monhystera* sp.	1	1	NT	NT	NT
		Prismatolaimus sp.	0	0	NT	NT	NT

1 Populations established.
0 Populations did not establish.
NT Not tested.

readily established by transferring individuals to fresh cultures of bacteria.

3.4 STYLET-BEARING SPECIES

Stylet bearing nematodes include the Tylenchida and most Dorylaimida. The host preferences of those species for which feeding relationships have been determined are frequently varied. To obtain an indication of the relative host specificity of stylet bearing nematodes from the Broken River fauna, a wide range of organisms was tested as potential hosts.

METHOD

Fungi: Isolates of Phoma* sp., Rhizoctonia solani† Kuhn, or Ulocladium atrum* Preuss, were cultured in Petri plates containing 1.0% water agar, or one-half strength Difco cornmeal agar at 20°C. The inoculated plates were incubated in continuous darkness, and inspected at weekly intervals. Feeding was observed through a cover glass lowered over nematodes on the surface of the cultures, or in glass ring chambers 17mm internal diameter by 9mm deep, sealed to a 22mm square cover glass and containing approximately 0.6ml of 1.0% water agar supporting fungus growth. In the latter instances the chambers were inoculated with the test fungus and incubated in an inverted position in closed Petri plates at 20°C until hyphae were observed ramifying over the surface of the cover glass, at which stage nematodes were introduced. Inversion of the cells in a Petri dish during subsequent incubation

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* Isolated from F. novae-zelandiae seed

† Isolated from psuedosclerotia of R. solani on potato tubers.

and over a glass micro-slide during examination minimised desiccation, but it was occasionally necessary to pipette a drop of cooled water agar into the cultures. By focussing through the cover glass, high power dry objectives and oil immersion objectives could be used to observe nematodes feeding on hyphae growing close to the undersurface.

Seedlings: Seeds of F. novae-zelandiae and L. pratense were surface sterilised by immersion for 10 minutes in a 4:1 solution of 1:1,000 mercuric chloride and 95% ethyl alcohol (Wood, 1966), rinsed in three changes of sterile distilled water, and placed on 1.0% water agar in 9cm diameter Petri plates. With the normal geotropic response, roots grew down through the agar and along the bottom of the plate. Surface sterilised nematodes were aseptically transferred into shallow troughs cut to about two-thirds of the agar depth and 3-5mm from the seedling root. Observations on nematode activity were made by inverting the Petri plates on a microscopic stage and focussing through the bottom.

Plant callus tissue: Lucerne (Medicago sativa L. variety Marlborough), callus tissue was grown on tissue culture media composed of salt solutions prepared according to White (1963), with vitamins and growth factors according to Krusberg's "improved" medium (Krusberg, 1961).

Lucerne seeds were surface sterilised by immersion in a 4:1 solution of 1:1000 mercuric chloride and 95% alcohol for 10 minutes, rinsed in three changes of sterile distilled water, and soaked in sterile distilled water for 24 hours. The seeds were then immersed in solution of 1.0% sodium hypochlorite for 5 minutes, rinsed in sterile distilled water, and placed in Petri plates containing 1.0% water agar. When the radicles of the germinated seeds were 1-2cm long, they were excised and

transferred to 250ml flasks containing approximately 200ml of culture medium. After two to three months incubation at 20°C in continuous darkness nematodes were placed on the callus growth, or on sections of callus tissue transferred to Petri plates containing fresh tissue culture medium. The inoculated cultures were incubated for four weeks at 20°C, dispersed in water, and examined for the presence of nematodes.

Algae: Mixed cultures of algae, bacteria and fungi, containing unidentified rhizopods and rotifers were prepared from a suspension of bottom material from a temporary fresh water pond, and dispersed in cooled 1.0% soil extract agar in 9cm diameter Petri plates. The most common algae present were, in decreasing order of abundance: Haematococcus sp., Microcoleus sp., and Chlorella sp. Feeding on Haematococcus was evidenced by the presence of the readily identified haematochrome in nematodes' intestines. Sections of agar containing Haematococcus with fungal and bacterial contaminants, but excluding nematodes, rotifers or other species of algae, were removed from the cultures and placed in glass ring observation chambers. Cooled soil extract agar was added and the cells were inoculated with adult nematodes from the initial cultures with haematochrome or chlorophyll in their gut. The inoculated chambers were incubated at room temperature under natural light, in an inverted position in Petri plates containing a thin layer of 1.0% water agar to prevent dehydration. Regular observations were made to detect the presence of nematode eggs and feeding juveniles.

Moss: Two species of mosses (Tortula princeps and Bryum sp.), were established in cornmeal agar soil plates. Sections from the filamentous thatch of protonemata were removed and washed repeatedly in sterile water before

inoculation onto soil extract agar. The cultures were incubated for one month at 16°C under artificial light before surface sterilised nematodes were introduced. Inoculated cultures were maintained at room temperature under natural light. Bacterial contaminants were invariably present, and fungi occurred in many plates. The suitability of moss protonemata as a food source was therefore based on the presence of chlorophyll in the intestines of nematodes, the presence of all stages feeding on protonemata, and from periods of continuous observation on the pattern of feeding of individual nematodes.

Bacteria: Nematodes were placed on mixed isolates of bacteria from Broken River soil, cultured on soil extract agar. The cultures were incubated at 24°C and examined periodically for evidence of feeding and reproduction.

Nematodes: Several eelworms were observed preying on other nematode species and nematode eggs in cornmeal agar soil plates. These, and other suspected predators were placed in Petri dishes containing populations of Aphelenchus avenae raised on the fungus U. atrum in one-half strength cornmeal agar, or Acrobeloides sp., cultured on E. coli. Regular observations were made to investigate the possibility of a predacious habit.

RESULTS AND DISCUSSION

From the results presented in Table 8 it is apparent that different species of Tylenchus Bastian, 1885 exhibit a wide range of food preferences. The taxonomy of the nematodes involved is discussed in Chapter 4. Although two of the species investigated are removed from Tylenchus to the closely related genera Aglenchus (Andrassy, 1954)

TABLE 8: Results of feeding trials with stylet bearing nematodes

Nematodes investigated

Order	Family	Species	Food Type						
			S	C	F	A	M	B	N
Tylenchida	Aphelenchoididae	*Aphelenchoides bicaudatus*	1	1	1	0	1	0	0
		Aphelenchoides sp.	0	NT	1	NT	NT	NT	NT
		Aphelenchus avenae	1	1	1	NT	1	0	0
		Seinura demani	0	NT	0	NT	NT	0	1
	Tylenchidae	*Aglenchus neozelandicus*	1	1	1	NT	1	0	0
		Cephalenchus tahus	1	NT	0	0	0	NT	NT
		Tylenchus maiakus	1	NT	0	NT	NT	NT	NT
		Tylenchus rikus	0	NT	1	0	0	NT	NT
		Tylenchus sp.	0	NT	1	0	1	NT	NT
		Ditylenchus sp.	0	NT	1	0	0	NT	NT
	Hoplolaimidae	*Helicotylenchus* sp.	2	NT	0	0	0	NT	NT
		Praylenchus penetrans	1	NT	0	NT	NT	NT	NT
	Neotylenchidae	*Deladenus durus*	0	NT	1	NT	0	NT	NT
	Cricenomatidae	*Paratylenchus projectus*	1	0	0	0	0	NT	NT
Dorylaimida	Dorylaimidae	*Eudorylaimus* sp.	0	NT	0	1	1	0	2
		Labronema sp.	0	NT	0	1	1	0	2
	Aporcelaimidae	*Aporcelaimellus paraamylovorus*	0	NT	0	1	1	0	2
		Aporcelaimus sp. (3)	0	NT	0	1	1	0	2
	Leptonchidae	*Tylencholaimus* sp.	0	NT	1	0	0	0	0
		Tylencholaimellus montanus	0	NT	1	0	0	0	0
	Nygolaimidae	*Nygolaimus* (*Paravulvus*) sp.	NT	NT	0	0	0	0	2
	Belondiridae	*Dorylaimellus* sp.	0	NT	0	0	0	0	0
	Diptherophoridae	*Diptherophora* sp.	0	NT	0	0	0	0	0

S = Seedlings
C = Lucerne callus tissue
F = Fungi
A = Algae
M = Moss
B = Bacteria
N = Nematodes

1 = Feeding and reproduction observed
2 = Feeding only observed
0 = No feeding or reproduction observed
NT = Not tested

Meyl, 1961, and Cephalenchus (Goodey, J.B., 1962)n. rank, in the present context they are considered with Tylenchus species to conform with previous discussion. Despite the common occurrence of Tylenchus species in the rhizosphere, very little has been reported on their feeding habits. Several workers have suggested that they probably feed on fungi or root hairs (Hirschmann, 1960; Seinhorst, 1961; Thorne, 1961), but until the present feeding trials were carried out the evidence has indicated that they are root parasites (Khera and Zuckerman, 1962, 1963; Sutherland and Keeble, 1966; Sutherland, 1967a, 1967b; Gowen, 1970). It appears that there may exist a range of preference from obligate fungus parasites to obligate higher-plant parasites with some intermediate facultative species; this would be similar to the range known to occur for species of the genera Aphelenchoides Fischer, 1894, and Ditylenchus Filipjev, 1934, (see Winslow, 1960; Thorne, 1961). Aglenchus neozelandicus (Egunjobi, 1967)n.comb., fed and reproduced on lucerne callus tissue and seedling roots in agar (Table 8). Feeding of adults on moss protonemata was observed, but populations did not establish. Post-hatch juveniles were observed to feed on fungus hyphae on two occasions, but did not survive to enter the second moult, and adults were never seen to penetrate fungi. Tylenchus sp., on the other hand, established populations on moss protonema and fungi and was seen to feed on seedling root hairs, while Cephalenchus tahus n.g. n.sp., and Tylenchus maiakus n. sp., are obligate higher plant parasites, and Tylenchus rikus n.sp., is an obligate fungus feeding species.

Several Aphelenchoides species are acknowledged facultative higher plant and fungus feeders, e.g.

A. fragariae (Ritzema-Bos, 1890) Christie, 1932, (Christie and Crossman, 1936), A. bessyi Christie, 1942, (Todd and Atkins, 1958), and A. blastophthorus Franklin, 1952 (recorded by Franklin (1952) on Scabiosa causica, and by Hooper (1963) on Botrytis cinerea Pers.). Similarly, Aphelenchus avenae Bastian, 1885, has been reported to feed on a range of fungi (Mankau and Mankau, 1963; Fisher and Edwards, 1967), and on the roots of higher plants (Chin and Estey, 1966). Nielsen (1949) suggested that A. parietinus (Bastian, 1865) Steiner, 1932, may feed on Chlamydomonas nivalis, and recently, Siddiqui and Taylor (1969), recorded the feeding of A.bicaudatus (Imamura, 1931) Filipjev and Schuurmans Stekhoven, 1941, on the fungi Pyrenochaeta terrestris de Not., Sporobolomyces sp., and Hanselula saturnus (Klocker)H. et P. Sydow, and on the green alga, Stichococcus bacillaris. The Broken River isolate of A. bicaudatus fed and multiplied on moss protonema, seedling roots and root hairs, and lucerne callus tissue in addition to fungi. The suitability of moss protonemata as a host is not surprising, as Aphelenchoides spp., are frequently prevalent in habitats characterised by growth of mosses (Winslow, 1960), but feeding on protonema has not been reported previously. Although Siddiqui and Taylor (1969), observed that the convexity of the host cell did not prevent A. bicaudatus from penetrating and feeding on small oval to spherical cells of Sporobolomyces sp. and H. saturnus, the Broken River isolate did not feed on the spherical cells of Haematococcus or Chlorella. Hence the main food organisms of the present isolates are considered to be filamentous forms. A. avenae exhibited a similar host range to A. bicaudatus, indicating that the nutritional requirements of these two species are similar. At least two species

of Aphelenchoides have been cultured previously on plant callus tissue: the obligate higher plant feeding species, A. ritzembosi (Schwartz, 1911) Steiner and Buhrer, 1932, (Krusberg, 1961; Dolliver, Hildebtandt, and Riker, 1962), and the fungus feeding species A. sacchari Hooper, 1958, (Meyer, 1967). As A. bicaudatus, which is intermediate between the two feeding types, also feeds on callus tissue it seems reasonable to suspect that the host range of many species of the genus may be less specific than previously considered.

The host preferences of the remaining species of Tylenchida investigated confirmed previous reports of the feeding habits of species of the genera represented (see Winslow, 1960; Thorne, 1961; Goodey, 1963; Southey, 1965).

The feeding habits of nematodes of the order Dorylaimida are not well known. Lindford (1937) reported that members of the genera Dorylaimus Dujardin, 1845, Discolaimus Cobb, 1913, and Actinolaimus Cobb,1913, were observed feeding on other nematodes and that Dorylaimus spp., feed on nematode eggs. Thorne (1939) considered Labronema Thorne, 1939, species to be predacious, but reported finding green chlorophyll-like material in the intestines of some animals, and Nielsen (1948) suggested that soil algae play an important role in the basic nutrition of Dorylaimus species. Later, Nielsen (1949) reviewed the early literature on the nutrition of free-living nematodes and commented on his own observations from the examination of gut contents of a large number of nematodes. He included most Dorylaimoidea in a group which he considered to be feeders on liquid, especially on plant juice. Hollis (1957) reported the culturing of Eudorylaimus etters-bergensis (de Man, 1885)Andrassy, 1959, on a range of

microorganisms which included a blue-green alga (Chroococcus sp.) green algae (Chlorella vulgaris Beijer., and Tetraedron sp.), a protozoan (Drepanomonas sp.) and a fungus (Cephalothecium sp.). It is apparent from Goodey (1963), that the bionomics of most species of the Dorylaimoidea are unknown, but that species of many genera are considered to be predacious and/or algae feeders. Esser (1963), recorded a predacious habit for nematodes of the genera Dorylaimus, Labronema, Discolaimus and Carcharolaimus Thorne, 1939. The prey included nematodes, oligochaetes, oligochaete eggs, a mite and a rotifer egg. Ferris (1968) confirmed the predacious habit of Labronema species in studies of the development of L. ferox Thorne, 1939, and L. thornei Ferris, 1968. All stages of L. ferox were reported to feed on nematodes or nematode eggs and occasionally on spores of fungi.

The wide host range of Eudorylaimus sp., and Labronema sp., recorded in the present trials (Table 8), demonstrate the relative non-specificity of their dietary requirements. However, neither species survived on seedling roots, bacteria, or fungus hyphae, nor was fungus mycelium necessary for the deposition of eggs as suggested by Ferris (1968). Aporcelaimellus paraamylovorus n.sp., and Aporcelaimus sp., also fed and multiplied on mixed algae cultures and on the protonema of moss, and were observed feeding on nematode eggs and nematodes (Table 8). In addition, A. paraamylovorus under observation in stock cornmeal agar soil plates has been seen feeding on enchytraeids, rotifers, and on one occasion, on the cadaver of a collembolan. Heyns (1965) considered that species of the Aporcelaimidae are usually predacious, feeding mostly on oligochaetes, but that Aporcelaimellus species may be predominantly vegetarian. The present data indicate that

they feed on both animal and plant material, with a host range similar to species of Dorylaimidae.

Tylencholaimus sp., reproduced on the fungi tested (Table 8), but no feeding on conidia, algae cells, or protonema was observed. Nielsen (1949) noted chlorophyll in the gut of Tylencholaimus miribilis (Butschli, 1873) de Man, 1876, and maintained the animals on water agar cultures of an unknown alga for six weeks. But during this period 10 females produced only three eggs (Nielsen, 1949), which suggests that nutrition was sub-optimal. Ferris (personal communication, 1970) cultured Tylencholaimus teres Thorne, 1939, on fungus mycelium. In view of this evidence and the evidence of the present feeding trials, species of the genus Tylencholaimus de Man, 1876, are considered to be primarily fungus feeders. Loof and Jairajpuri (1968) transferred Tylencholaimus from the Dorylaimidae to the Leptonchidae. Siddiqui (1969) raised the sub-family Tylencholaiminae to family rank and included Tylencholaimus in it. Similarly, Tylencholaimellinae was given family status and Tylencholaimellus M.V. Cobb, 1915, was transferred from the Leptonchidae to Tylencholaimellidae. In the present trials, Tylencholaimellus montanus Thorne, 1939, was shown to be a fungus feeding species with similar biology to Tylencholaimus n.sp. This may be indicative of a closer relationship than that considered by Siddiqui (1969).

The predacious habit of Nygolaimus spp., was recognised by early workers (Cobb, 1929; Thorne, 1930), but they are generally considered to prey on small oligochaetes (Thorne, 1961; Goodey, 1963). On one occasion a large population of Nygolaimus (Paravulvus) sp., was established on a corn-meal agar soil plate containing Rhabditida and Araeolaimida but devoid of oligochaetes. As the plates were "rejuvenated"

by monthly applications of fresh cornmeal agar, a continued supply of prey was ensured which enabled the predator to increase in numbers over several months. Feeding was observed on nematodes and nematode eggs. Large nematodes and moulting nematodes were most frequently killed, and cannibalism was noted in three instances. Nygolaimus (Paravulvus) sp., placed on Haematococcus did not survive, but one adult was observed with a small amount of haematochrome in its intestine which indicated that at least one algal cell was ingested.

Attempts to culture Dorylaimellus sp., and Diphtherophora sp., were unsuccessful. This may have resulted from a failure to meet specific host requirements or from the effects of unfavourable culture conditions. In some instances, adult female Dorylaimellus sp., laid fertile eggs, but on no occasion was feeding observed, and juveniles failed to mature.

3.5 GENERAL DISCUSSION

It is apparent that different species of nematodes exhibit a wide range of food preferences, that several species are catholic in their feeding habits and, further, that species from the same or closely related genera may occupy different ecological niches in the soil.

The nutrition of non-stylet-bearing nematodes of the Rhabditida and Araeolaimida from Broken River is associated with the presence of bacteria although it has not been positively demonstrated whether bacteria or the products of their metabolism are the nutrient source. However, Nielsen (1949) considered that there was no doubt that nematodes feed on bacteria even if accessory food substances were utilised. Observations on nematodes in cornmeal agar soil plates indicates that feeding is an indiscriminate process, ingestion being limited only by

particle size. By this means, bacteria, organic and inorganic compounds associated with the presence of bacteria, other microorganisms, and small particles of detritus in suspension may be ingested. Jensen (1967) showed that Mesodiplogaster lheritieri (Maupas, 1919), Goodey, 1963, ingest spores of Fusarium oxysporum Schlecht.ex Fr., and Verticillium dahliae. The same author suggested that Panagrolaimus subelongatus (Cobb, 1914) Thorne, 1937, Panagrellus redivivus (Linnaeus, 1767) Goodey, 1945, and Rhabditis spp., also ingest fungus conidia during feeding. Hence it appears that extra-oral selection of food material is of minor consequence during feeding of many non-stylet-bearing nematodes; where host specificity occurs, it is probably invoked during the digestive processes.

Stylet-bearing nematodes feed on a wide range of plant and animal foods. Nielsen (1949) considered that the presence of a stylet indicates that the chief food is obtained by sucking. Ingestion was preceded by penetration of the host cell wall with the nematode spear and coincided with pulsation of the oesophageal gland apparatus for all species of Dorylaimida and Tylenchida (Deladenus durus (Cobb, 1922) Thorne, 1942 excluded) cultured in the present study. Feeding was not observed unless penetration was achieved. Species of Tylenchida from Broken River are primarily plant parasites, but one predator (Seinura demani (Goodey, 1928) J.B. Goodey, 1960) occurred. Food plants include fungi, mosses and higher plants. The degree of food specialisation varies; some species are obligate fungus or higher plant feeders, others are facultative plant feeding forms. The Dorylaimida on the other hand contains obligate fungus feeding forms (Leptonchidae), species which parasitise both plant and

animal cells (Dorylaimidae and Aporcelaimidae), and obligate predators (Nygolaimidae). Thorne (1930) noted that both "nygolaims and sectonemas" refused to devour other nematodes, but attacked and fed on oligochaetes. Although all of the predacious Dorylaimida cultured in the present trials attacked and feed on other nematodes, the proportion of kills relative to attacks was low. When stimulated by contact with a nematode spear prey animals move rapidly away. Croll and Smith (1970) reported a similar sensitivity in Rhabditis sp., to peripheral and mechanical stimulation. As a consequence, large animals (including other Dorylaimida), moulting animals and nematode eggs were most frequently killed.

4. SPECIFIC BIOLOGICAL STUDIES

4.1. INTRODUCTION

Knowledge of the biological aspects of nematode ecology is fundamental to the interpretation of distribution patterns of nematodes, of fluctuations of numbers in population dynamics studies, and the interpretation of the role of nematodes in plant disease. With the multiplicity of factors operating in an environment as heterogeneous as the soil, studies of the biology of nematodes, like many soil organisms, involve complex problems. However, the development of culturing techniques in the laboratory has minimised many of the problems by facilitating the observation of animals under conditions in which the number of variables involved at any one time can be controlled. As it was impracticable to consider all of the species present at Broken River in detail, a number of species representing a range of feeding types and taxonomic groups were selected for detailed studies. To develop the understanding of interrelationships between nematodes and the various components of the soil fauna and flora, emphasis was placed on life-histories, host ranges, and host/parasite interactions. Where necessary, the systematics of particular species are discussed in conjunction with considerations of their biology.

The influence of other soil organisms on nematodes is not confined to feeding relationships. Of the many organisms implicated in interactions, nematode-trapping fungi have received considerable attention. Investigations on the frequency of occurrence and host-parasite relationships of nematode predacious fungi in the Broken River rhizosphere were carried out in view to considering the

effects of their presence on the distribution pattern of nematodes.

4.2 GENERAL METHODS

Methods used throughout the chapter are discussed. Those methods specific to particular experiments are detailed in the appropriate sections.

Figures: Photomicrographs were recorded on 35mm Ilford Pan F film (ASA 125) using a Zeiss Standard GFL microscope with a Zeiss attachment camera. Drawings were made using a Leitz Orthoplan microscope with drawing attachments.

Morphology: Specimens were examined alive in temporary water mounts, in glycerine (Seinhorst,1959), after staining in acetic orcein (Hirschmann, 1962), or mounted in 5% formalin containing acid fuschin (Hechler and Taylor, 1966a).

Unless otherwise stated, measurements of adults were made on animals relaxed by gentle heat, fixed in TAF (Courtney, Polley and Miller, 1955), processed to glycerine according to Seinhorst (1959), and mounted following the method of Goodey (1963a). Measurements of juveniles were made on animals relaxed by heat and fixed in TAF. Gonad development and details of gonad structure were observed in animals stained in acetic orcein, and animals mounted in formalin containing acid fuschin.

Cuticular structures of the Tylenchidae were observed on nematodes fixed in TAF and mounted in Shillaber's non-drying immersion oil, Grade 'B' high viscosity. Although contraction occurred after a few hours, the technique proved admirable for demonstrating the pattern of the lateral fields and cuticular annulation for photographic and counting purposes (see Figures 9 and 11).

En face mounts were prepared using the decapitated heads of nematodes which had been processed to glycerine. The heads were set in a vertical position in a film of cooled (about 45°C) 1.0% water agar and observed through a cover-glass gently lowered over the lips.

Observations on the position and form of the vulva and anus were made on fixed animals placed on the surface of a film of water agar along with fine glass spacing rods of about the same diameter as the nematodes. A cover-glass was lowered over the nematode and by careful manipulation of the cover-glass the body may be rolled over to bring the required surface into view.

Biology: Observations on life-histories, host ranges and host-parasite relationships were made using Petri plate cultures, or cultures in glass ring observation chambers (see Chapter 3). Surface sterilisation methods and general procedures were the same as for Chapter 3.

Several potential food organisms were tested in host range studies carried out for specific nematodes. Seeds of pasture plants were obtained from the Government Seed Testing Station, Palmerston North, and supplemented with seeds collected from plants at Broken River. Fungi and bacteria were obtained from the Department of Microbiology and Genetics, Massey University, Palmerston North, supplemented with isolates from the Broken River rhizosphere. Algae were supplied by Dr. Flint of the Botany Department, University of Canterbury.

Detailed observations on the developmental biology of nematodes feeding on fungi or algae were made using glass ring culture chambers. Water agar or soil extract agar were used to minimise the effects of contamination which sometimes occurred during long periods of observation. Fungi were inoculated onto the surface of the

medium in the cells which were then incubated in an inverted position over a layer of moist sterile filter paper in closed Petri plates at 20°C. The plates were incubated until hyphae were observed ramifying over the surface of the cover glass, at which stage surface sterilised nematodes were introduced. Concentrated suspensions of algal cells harvested from stock cultures grown on Czurda's medium (Ettl, 1959) under artificial light at 16°C, were placed in the bottom of the culture cells. Cooled soil extract agar was added and on solidification of the medium, nematodes were introduced. The inoculated culture cells were maintained at 18-20°C in Petri plates containing sterile moistened filter paper.

Studies on nematodes feeding on plant roots were made in Petri plates containing 1.0% water agar supporting the growth of perennial ryegrass, short rotation ryegrass, or fescue tussock seedlings. Surface sterilised seeds were germinated in water agar, and when the radicle was about 0.5-1.0cm long the Petri plate was inverted. With a normal geotropic response, the roots grew downward toward the inverted surface of the medium. A cover-glass was placed over the root tip causing subsequent growth to continue in close contact to the glass. Surface sterilised nematodes were introduced and feeding could be observed at high magnifications by focussing through the thin cover-glass.

Three or more transfers were sometimes necessary to follow individuals of long-lived species through a generation.

The effect of temperature on the time required for egg hatching was estimated for most species. The minimum temperature at which hatching occurred was taken as the approximate limiting temperature for reproduction. The

time required for eggs to hatch at 5, 10, 15, 20 and 25°C was recorded. Five to 15 gravid adult nematodes were added to glass ring culture chambers containing the appropriate food organism. The adults were removed after 24 hours in the case of short lived species, or 48 hours in the case of long lived species, a small amount of cooled water agar was added to the surface of the cells and the position and number of eggs located close to the cover-glass recorded. For the 5, 10 and 15°C treatments adults were obtained from cultures previously incubated at 15°C; for the 20 and 25°C treatments adults were incubated at 20°C prior to extraction. Regular observations were made to determine the number of eggs which hatched at each temperature.

4.3 PARATYLENCHUS PROJECTUS JENKINS, 1956

INTRODUCTION

Paratylenchus Micoletzky, 1922, species frequently occur in large numbers in the rhizosphere (e.g. Ward, 1960; Faulkner, 1962, 1964; Sutherland, 1965; Brown, Palmiter and Keplinger, 1966). Aspects of the biology of several species have been reported (e.g. Lindford, Oliviera and Ishii, 1949; Ruever, 1959; Rhoades and Lindford, 1959, 1961a, 1961b; Paracer, 1966; Fisher, 1966, 1967), and some species have been shown to cause reductions in plant growth (e.g. Lownsberry, Stoddard and Lownsberry, 1952; Faulkner, 1962, 1964; Fisher, 1967).

P. projectus was isolated in large numbers from Broken River soil. The life-history, host-parasite relationships and host range of P. projectus have been discussed (Coursen, Rhode and Jenkins, 1958; Coursen and

Jenkins, 1958; Norton, 1959; Rhoades and Lindford, 1959, 1961a, 1961b; Sutherland, 1967), but the significance of the nematode in causing losses of dry matter production of pasture has not been established. A slight stunting of tall fescue, associated with increased tillering and root proliferation has been attributed to parasitism by P. projectus (Coursen and Jenkins, 1958), whereas Norton (1959) reported that this species did not cause economic losses to wheat plants under field conditions.

Aspects of the biology of P. projectus were studied as a pre-requisite to plant pathological considerations. In view of previous studies, investigations on feeding and life-history were limited; emphasis placed on testing some of the common pasture species at Broken River as potential hosts for P. projectus.

METHOD

Host range: Surface sterilised seed of white clover, suckling clover, perennial ryegrass, crested dogstail, yorkshire fog, timothy, goosegrass, sweet vernal, browntop, cocksfoot (Dactylis glomerata L.), fescue tussock and chewing fescue (Festuca rubra var. fallax Hack.) was sown in 9.5 x 10.0cm pots containing heat sterilised soil. Four weeks after sowing the plants were thinned to 10 per container, and three weeks later two pots for each species were inoculated with 100 surface sterilised P. projectus. Because of their relatively slow growth rate, fescue tussock seedlings were grown for 10 weeks before inoculation. Six months after inoculation nematode population levels in each of the pots were determined. Soil cores were taken through the centre of the foliage to the bottom of the pots and the nematodes extracted using the methods described in Chapter 2.

RESULTS

MEASUREMENTS: Fourth stage juveniles (n = 10): L = 270-340μ (310); a = 16.6-19.8 (17.7); b = 4.1-4.5 (4.3); c= 11.9-15.5 (13.8); stylet (tip) = 10.0-13.0μ (11.5).

Females (n= 10): L = 328-445μ (395); a = 20.5-25.4 (23.8); b= 3.1-4.1 (3.6); c= 14.2-17.9 (15.2); V = 84.4-86.8% (85.5); stylet = 28.0-29.5μ (28.5).

PENETRATION AND FEEDING: Root penetration is achieved by repeated thrusts of the stylet (about one thrust per second) against the epidermal cell wall. The process is interspersed with short periods of inactivity and continues until penetration is achieved up to two hours after its initiation. About 30 minutes elapse before pulsation of the median oesophageal bulb occurs. During feeding, a dome of granular material forms within the cell around the orifice of the stylet tip. Nematodes were seen to feed only on epidermal cells (Figure 7), sometimes remaining at one site for up to six days.

LIFE-HISTORY: Females laid one to three eggs per day. Because the animals are sedentary during long feeding periods, the eggs are deposited in clusters. Hatching occurred after 7-8 days in 1.0% water agar at 18-20°C, and the time for a complete generation (egg to egg) under these conditions was 36-38 days (four observations).

HOST RANGE: Populations of P. projectus were harvested from all of the hosts tested (Table 9). Non-feeding fourth stage pre-adult juveniles characteristic of P. projectus (Rhoades and Lindford, 1961a) comprised about 50% of most populations.

FIGURE 7. *Paratylenchus projectus* feeding on epidermal root cells of perennial ryegrass.

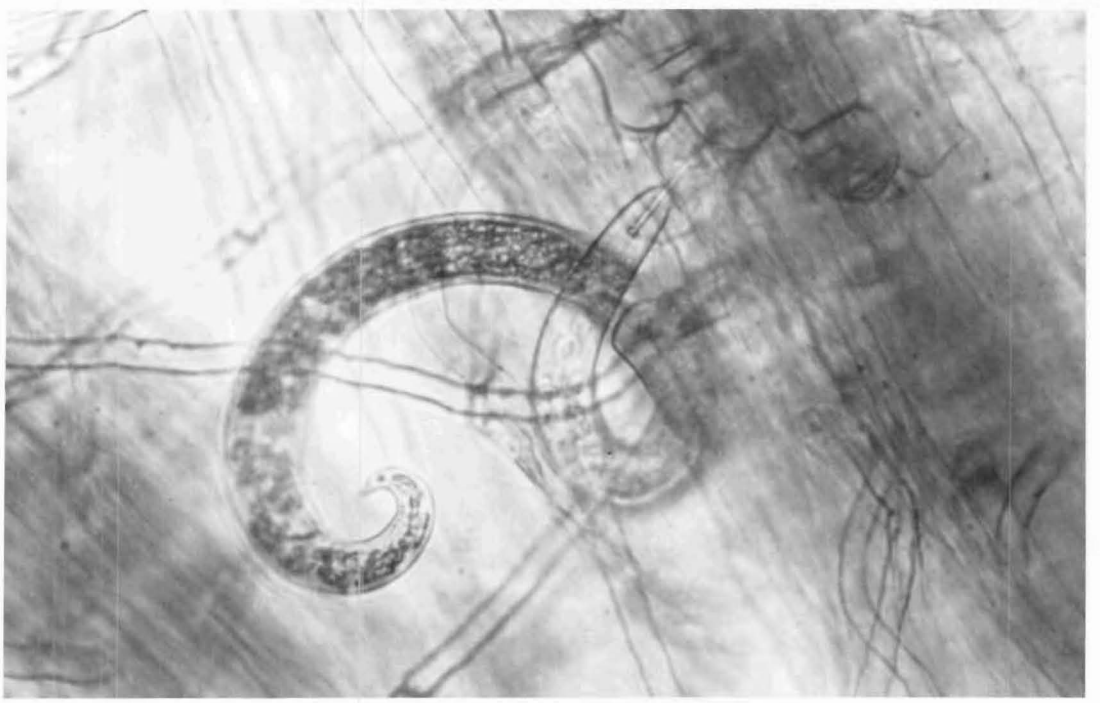


TABLE 9: Populations of P. projectus established on seedlings grown under green-house conditions in host range trials. Each pot contained 10 seedlings of one of the species listed below, and was harvested six months after inoculation with 100 nematodes.

Plant species	Number of nematodes per ml of soil.		
	Pot 1	Pot 2	Mean
White clover	65.6	51.4	58.5
Suckling clover	50.3	28.5	39.4
Perennial ryegrass	140.4	190.5	165.4
Crested dogstail	70.6	79.5	75.0
Yorkshire fog	130.5	50.8	90.6
Timothy	85.8	129.0	107.4
Goosegrass	70.3	49.3	58.8
Sweet vernal	88.8	130.4	109.6
Browntop	45.6	68.2	56.9
Cocksfoot	112.5	121.4	116.9
Fescue tussock	50.6	42.3	46.4
Chewings fescue	29.4	86.6	58.0

DISCUSSION

Observations on penetration and feeding confirm the results obtained by Rhoades and Lindford (1961a, 1961b), although feeding on root hairs was not seen in the present study. The generation time in water agar at 18-20°C was five to eight days longer than that time recorded by Rhoades and Lindford (1961a) at 25-28°C. These workers reported that the life-cycle of P. projectus was reduced by seven to eight days in soil; a reduction of a similar order may be applicable to the present populations.

Comparisons of host suitability were not made for two reasons; firstly, the amount of root produced by each plant species was not determined; secondly, the ceiling population level for P. projectus on each of the species was not demonstrated. However, the wide host range recorded for P. projectus indicates that the distribution pattern of plant species is unlikely to have a direct influence on the distribution of the nematode. If root parasitism by P. projectus is detrimental to plant growth, the effects of feeding may be widespread in a close sward of pasture plants.

4.4 TYLENCHIDAE

Species of the family Tylenchidae are frequent components of populations of soil nematodes. Their presence in New Zealand soils was noted by Egunjobi (1967, 1968a, 1968b). Several species of Tylenchidae were isolated from Broken River. Because of the paucity of information on the biology of these nematodes, and their abundance at Broken River, the systematics of the most common species were considered, and a plant feeding form and a fungus feeding species selected for further study.

4.4.A Taxonomy

Since Andrassy (1954) reviewed the genus Tylenchus and established the four subgenera Tylenchus, Aglenchus, Filenchus and Lelenchus within it, there have been several opinions expressed regarding the taxonomic status of Tylenchus species. Thorne (1961), did not recognise Andrassy's subgenera as he considered that the characters used to delimit the groups frequently overlapped. On the other hand Meyl (1961), raised the status of the subgenera to generic rank. Goodey (1963), retained the subgeneric ranking and included Miculenchus Andrassy, 1959, as an additional subgenus. In 1963, Andrassy confirmed the position of Aglenchus (Andrassy, 1954) Meyl, 1961, as a separate genus. Three further subgenera have been added since Goodey's (1963) publication: Cephalenchus (Goodey, 1962); Clavilenchus (Jairajpuri, 1965), and Ottolenchus (Husain and Khan, 1967). Egunjobi (1968a) considered that the differentiating characters for Aglenchus were not sufficient to justify a generic rank, and retained Goodey's (1963) subgeneric status for the group (Egunjobi, 1967, 1968), but Szczygiel (1969) was of the opinion that Aglenchus constituted a valid genus. Thorne and Malek (1968), accepted Aglenchus as a genus and also gave Clavilenchus a generic status, but the subgenera Tylenchus, Filenchus, Lelenchus, Miculenchus, Cephalenchus and Ottolenchus were not recognised. However, Geraert (1968), redefined the subgenus Cephalenchus and included two species under it. Wu(1969), considered that the characters used to separate Tylenchus, Filenchus and Lelenchus were unreliable, and as a consequence, assigned new species to the genus Tylenchus without regard to subgeneric designation, but in a later paper (Wu, 1970), raised Ottolenchus to generic rank.

I agree with Andrassy (1963), Thorne and Malek (1968)

and Szczygiel (1969) on the generic status of Aglenchus, and accept the presence of lateral vulval membranes as a basic diagnostic character for the genus. In keeping with this decision and on the basis of observations of a previously undescribed tylenchid, together with the observations reported by Gaerert (1968), Cephalenchus is also considered to warrant generic rank. However, separation of Tylenchus, Lelenchus and Filenchus is less well defined. Therefore, following the lead of Wu (1969), the remaining common tylenchids from Broken River are assigned to Tylenchus without regard to subgeneric status until more stable differentiating characters are found.

4.4.A.I Genus Cephalenchus n. rank.

Syn. Tylenchus (Cephalenchus) Goodey, 1962.

Diagnosis.

Tylenchinae. Small nematodes. Body with coarse annules, head offset by a constriction, annulated. Six lateral field incisures. Vulva right subventral in position, not symmetrical, with lateral flaps. Post vulval uterine sac large and well developed. Bursa assymetrical, left ala usually shorter than the right.

Type species: Tylenchus emarginatus Cobb, 1893 (= Cephalenchus emarginatus (Cobb, 1893) n.comb.). Syn. Tylenchus hexalineatus Geraert, 1962. Tylenchus (Cephalenchus) megacephalus Goodey, 1962.

Other species: Cephalenchus leptus (Siddiqui, 1963) n.comb. Syn. Tylenchus (Cephalenchus) leptus Siddiqui, 1963.

4.4.A.II Cephalenchus tahus n. sp. (Figure 8 A-G; Figure 9 A-D)

Holotype female: L = 612μ; a = 37.9; b = 6.1; c = 3.8; v = 6.2%; G_1 = 45.0%; G_2 = 3.8% spear = 14.5μ.

Allotype male: L = 615μ; a = 47.3; b = 6.3; c = 4.3;

T = 76.6%; G = 55.3%; spear = 16.5μ.

Paratypes (25 females): L = 560-657μ (606); a = 33.0-48.7 (39.7); b = 5.7-6.9 (6.2); c = 3.4-4.6 (4.0); v = 59.6-67.7% (62.7); spear = 14.0-16.0μ(14.9). 3 males: L = 538-613μ (571); a = 31.6-36.0 (33.6); b = 5.8-6.2 (6.0); c = 4.4-5.2 (4.7); T = 76.8-80.8% (78.3%); spear = 16.5-18.0μ (17.3).

Females: Body filiform, cuticle coarsly annulated (annules 1.0-1.5μ wide at the level of the median oesophageal bulb, and 2.0-2.5μ wide immediately posterior to the vulva), tapering slightly from about the oesophago-intestinal junction, and markedly attenuated posteriorly from near the vulva (Figure 8G). Sub-cuticular annulation distinct, usually appearing finer than that of the cuticle, particularly in the region of the oesophagus and the tail. Lateral fields interrupting the transverse striations about a half-body-width across, marked by six incisures (Figure 8C; 9B), over about 60% of the body length, outer ones crenate. Incisures originate between the 14th and 17th annules, usually as two lines, increasing to three on the next posterior annule (Figure 9A), but variations may occur. The central incisure branches at about the 28th annule, and the resultant pair of incisures branch again 15-20 annules further behind, frequently appearing as a broken line for a distance of four to five annules. Deirids appear as a faint pore located in the middle of each lateral field just posterior to the excretory pore. Hemizonids were not observed. The incisures reduce to four, one to two anal-body-widths anterior to the anus, to three, six to seven anal-body-widths posterior to the anus, terminating a further anal-body-width toward the tail terminus.

Head rounded, offset by a constriction from the body,

FIGURE 8. *Cephalenchus tahus* n. sp.
A) Oesophageal region.
B) Lateral view of female sex organs. C) Lateral view of vulval region-cuticular structures.
D) Ventro-lateral view of vulval region-cuticular structures.
E) Lateral of male sex organs.
F) Entire female.
G) Entire male.

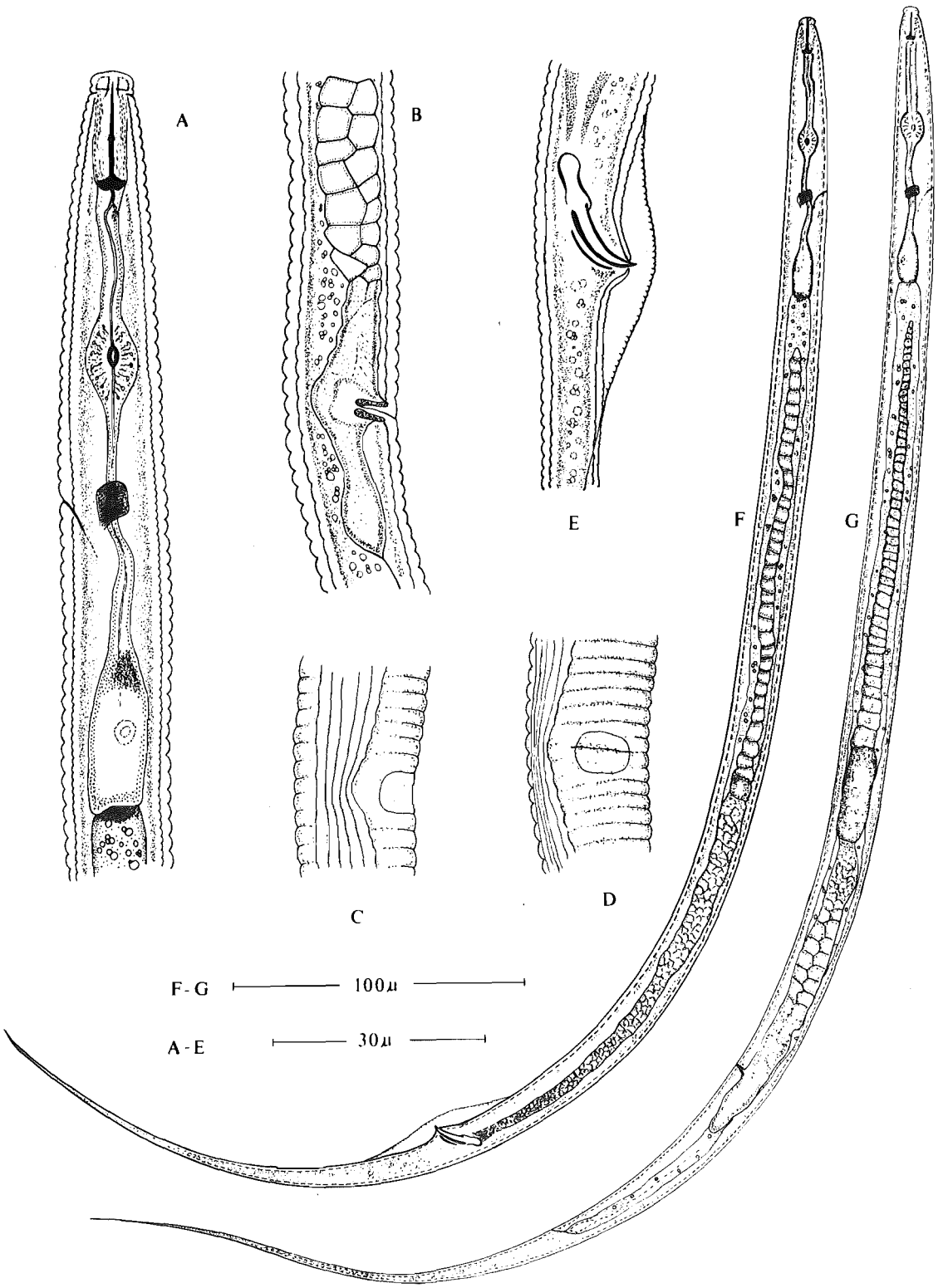
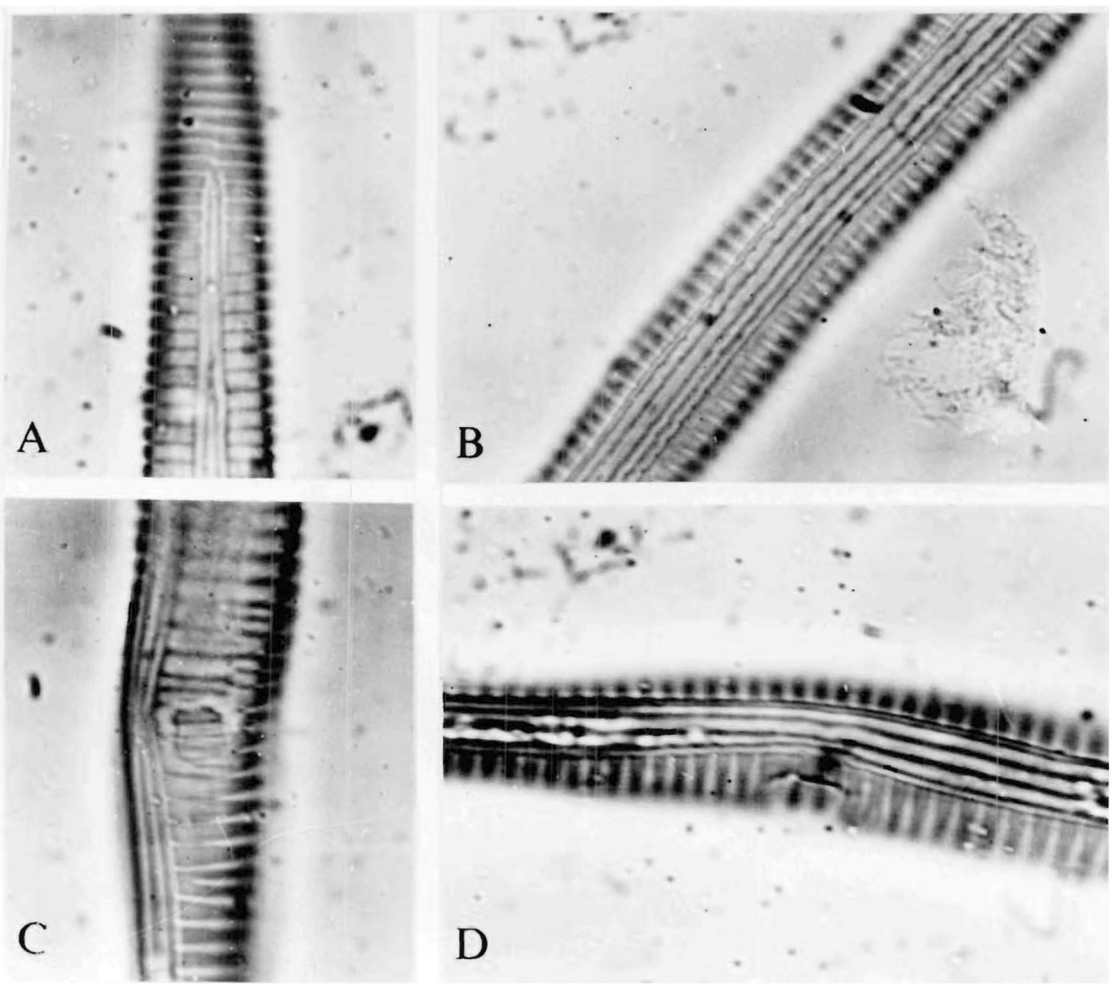

A
B
C
D
E
F
G
F-G 100μ
A-E 30μ

FIGURE 9. Cuticular structures of Cephalenchus tahus. A) Origin of lateral fields - oesophageal region. B) Lateral fields at mid-body. C) Ventro-lateral view of vulval region. D) Lateral view of vulval region.


A
B
C
D

with two annules.

Stylet slender, about 15μ long, the anterior end about 40% of the total length; basal knobs well developed, measuring 3μ across, margins anteriorly concave and posteriorly convex. Dorsal oesophageal gland orifice about 2μ from the base of the stylet (Figure 8A).

Procorpus of the oesophagus relatively wide. Median oesophageal bulb oval, about 11μ long and 6μ wide, with crescentic valve plates, about 3μ long located in the centre (Figure 8A). Isthmus slender, about 30% of the oesophagus length, enveloped by the nerve ring slightly posterior to its middle. Excretory pore opposite the nerve ring, usually towards the posterior end, at 55-60% of the oesophagus length. Terminal oesophageal bulb elongate, sac-like, measuring about 25 x 6μ , containing a granular refractive area in the anterior half, a prominent gland nucleus just posterior to the mid-point, and usually abutting squarely at the oesophagu-intestinal junction (Figure 8A).

Vulva right sub-ventral in position. Vulval aperture at base of a depression, a transverse slit 8-9μ across with slightly plicated lips. Lateral extremities of the vulva covered by a cuticular flap, leaving an opening of about 5 x 6μ . The unusual location of the vulva is associated with a constriction of the lateral field on the right side of the body (Figures 8C, D; 9C, D). Vagina strongly thickened, angled slightly anteriad; gonad outstretched, uterus consisting of a short muscular section leading to a section of large uterine-wall cells, usually two cells deep and about nine cells long, then to a short spermatheca, constricted at the oviduct, with one ovary consisting of 40-50 oocytes arranged in single file; postuterine sac well developed, 18-24μ long (Figure 8B, G).

Males: Males of similar shape to females, but stylet length is 2-3μ longer. Testis single, outstretched, 35-40 spermatocytes arranged in single file comprise about 50% of the gonad length (Figure 8F). Spicules paired, about 20μ long, typically tylenchoid, ventrally arcuate, pointed at the tip (Figure 8E). Gubernaculum curved, about 5μ long. The bursa is adanal, the right ala is about four anal-body-widths long with a crenate edge, the left ala is frequently reduced, three to four anal-body-widths long crenate.

Type locality: From soil around roots of Festuca novae-zelandiae at Broken River (map reference N.Z.M.S. 1, Sheet S66); altitude, 720 m.s.l.

Diagnosis: Cephalenchus tahus is characterised by its offset head; coarse annulation; the shape of the spear knobs; its recessed vulva with lateral membranes; the assymetrical location of the vulva; the inconsistent form of the right bursal ala, and the possession of six lateral field incisures. C. tahus differs from both other Cephalenchus species by the larger number of oocytes in the ovary (about 20 in C. emarginatus (Goodey, 1962), and 19 in C. leptus (Siddiqui, 1963); 40-50 in C. tahus), and the well defined vulval flaps. It differs further from C. leptus by the more posterior location of the vulva and the presence of males; and from C. emarginatus by the shape of the spear knobs.

The specific epithet 'tahus' is derived from the Maori word 'taha', meaning side, and refers to the unusual lateral location of the vulva in females.

4.4.A.III Aglenchus neozelandicus (Egunjobi, 1967) n. comb.

(Figure 10A - E;
Figure 11 A - D).

Syn. Tylenchus (Aglenchus) neozelandicus Egunjobi, 1967.

Tylenchus (Aglenchus)neozelandicus was described by Egunjobi (1967), from soil from under the canopy of an apple tree (Malus pumila (Mill)cv. Sturmer), Batchelar Orchard, Massey University, Palmerston North, New Zealand. A nematode closely resembling Egunjobi's (1967) description of T. (A) neozelandicus was isolated from the Broken River site. Examination of the type material of T. (A) neozelandicus revealed that the Broken River isolate was the same species, but that both the type material and the present isolate differed in some characteristics from the original description. The following is an ammended description based on observations of the type material and specimens collected in the present study.

Holotype female (Egunjobi, 1967): L = 0.51mm; a = 38.2; b = 5.4; c = 2.7; G_1 = 18.6% V = 82.5%; spear = 17μ .

Holotype female (remeasured): L = 486μ ; a = 25.6; b = 5.7; c = 5.3; G_1 = 18.5%; V = 66.46%; spear = 11.0μ .

Additional specimens from Broken River, n= 16: L = 440-505μ (468); a = 24.4-34.1 (28.5); b = 5.3-6.1 (5.7); c = 4.8-5.8 (5.2); G_1 = 21.8-32.0% (26.4); V = 62.6-70.0% (64.9); spear = 10-12μ .

Body filiform, cuticle coarsely annulated; annules about 2.5μ wide at mid-body, broken by longitudinal ridges which give a block-like cuticular sculpture (Figure 10C; 11A). Head narrower than body, about 3μ high and 6μ wide, with four annules. Amphids with strongly sclerotised pouches. Fourteen lonitudinal ridges initiate just behind the head, continuing to about six annules anterior to the vulva where a ventro-lateral ridge on each side of the body terminates abruptly (Figure 11B); about five to six annules posterior to the vulva, the ventral ridges combine (Figure 11C). Subsequent combination and

FIGURE 10. Aglenchus neozelandicus n.comb.
A) Entire female. B) Oesophageal region. C) Block-like cuticular sculpture. D) Lateral field.
E) Female genitalia and gonad.

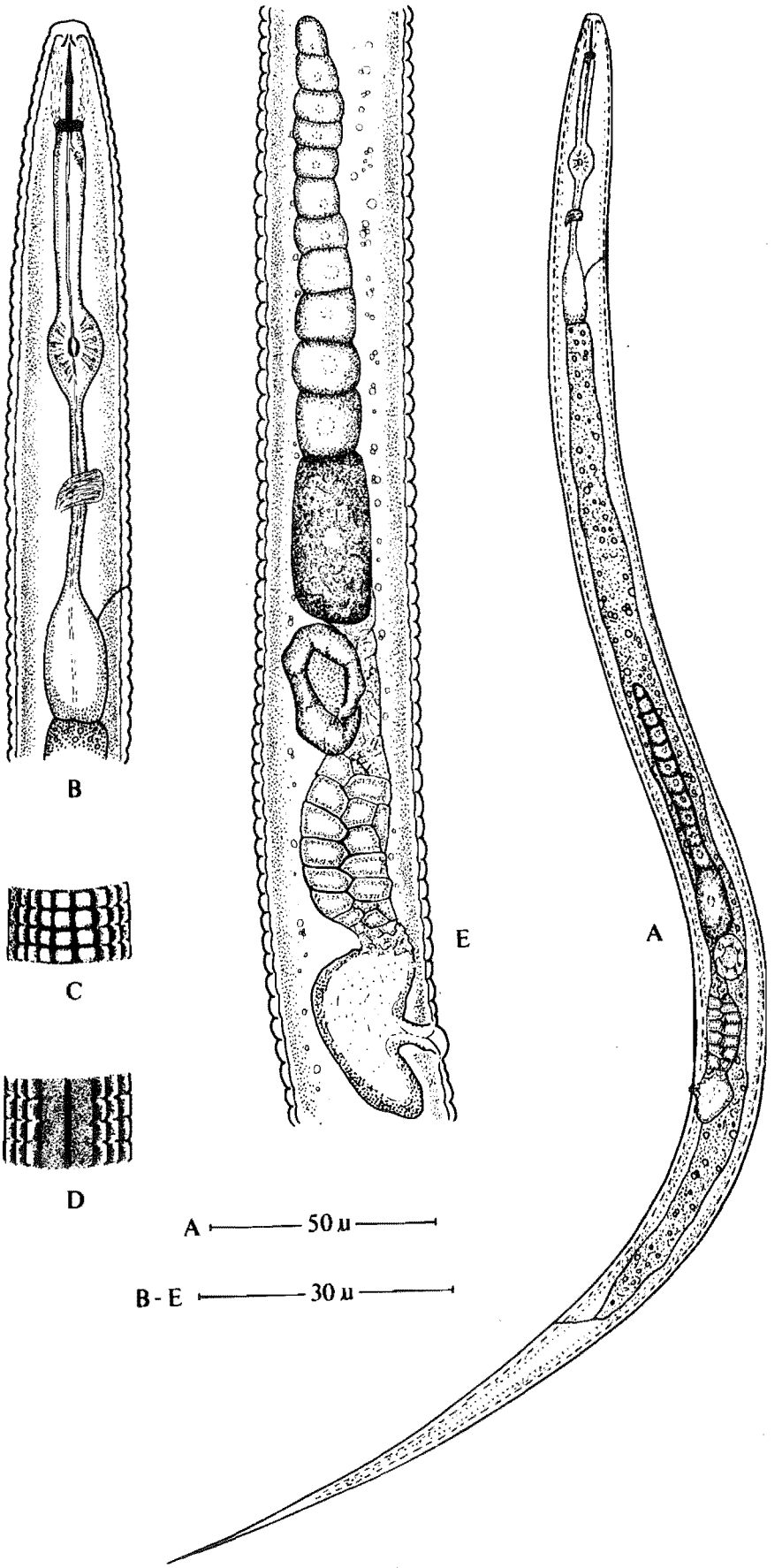
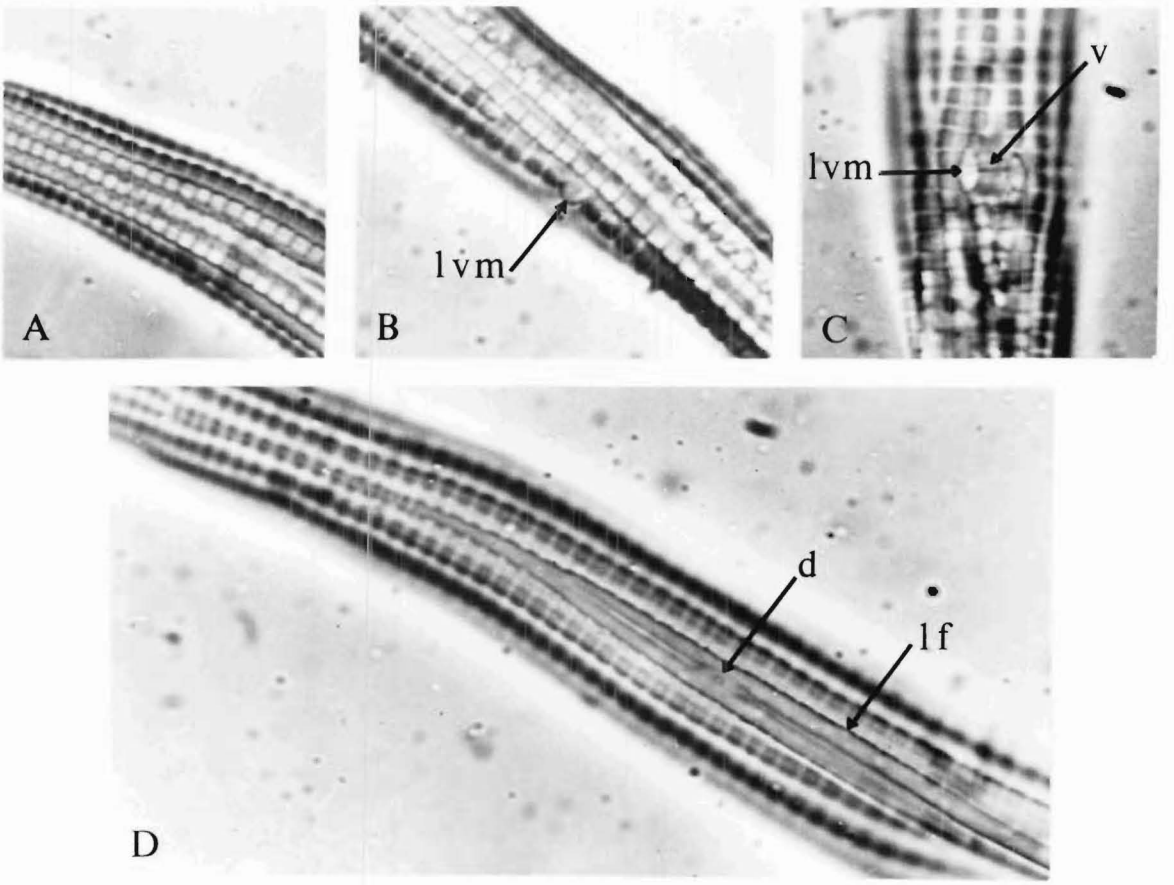

B
C
D
E
A
A 50 µ
B - E 30 µ

FIGURE 11. Cuticular structures of Aglenchus neozelandicus.
A) Cuticular sculpture-oesophageal region. B) Vulval region-lateral view (lmv=lateral vulval membrane). C) Vulval region-ventral view (v=vulva). D) Origin of lateral field-oesophageal region (d=deirid; lf=lateral field).


A
B
lvm
C
v
lvm
D
d
lf

termination of the ridges results in their disappearance about four to five anal-body-widths anterior to the tail terminus. Tail about 11 anal-body-widths long, annulated to the tip, usually with a fine point (Figure 10A), but sometimes shorter and abruptly terminated.

Lateral field one-quarter to one-third the greatest body diameter, with three distinct incisures, outer ones crenate (Figure 10D). Incisures begin as two about 22 annules behind the head, and become three, six to 12 annules posteriorly (Figure 11D). Deirids, present in each lateral field, interrupt the middle incisure about 7-8 annules posterior to its point of initiation (Figure 11D). About the mid-body region the central incisure may appear as two fine lines about 1.0μ apart, but in cross section can be seen as a central flat area between two longitudinal ridges.

Stylet well developed, about 11μ long with prominent rounded basal knobs about 2.5μ across; anterior conical section about 45% of the total length. Dorsal oesophageal gland orifice about 1μ from the base of the stylet.

Procorpus of oesophagus relatively wide. Median oesophageal bulb oval, about 10μ long and 8μ wide, with crescentic valve plates located in the centre. Isthmus slender, about 30% of the oesophageal length, enveloped by a nerve ring at approximately the mid-point. Excretory pore opposite, or just behind the anterior end of the terminal oesophageal bulb, elongate to oval, sac-like, measuring about 18 x 18μ (Figure 10B).

Vulva a transverse slit, about 6μ wide, located in a depression, lateral extremities beneath prominent lateral vulval membranes, anterior and posterior margins of the lips plicated. Vagina strongly thickened, with a short muscular uterus leading to a section of large cells usually

two deep and about seven cells long; posterior uterine branch short. Spermatheca, a diverticulum just posterior to the oviduct, leading to a single ovary, outstretched, composed of a single row of oocytes, usually about 12-14 (Figure 10E).

Discussion: I am satisfied that the Broken River material is conspecific with that described by Egunjobi. Ammendment to Egunjobi's (1967) description was considered necessary in view of the inconsistency between the reported measurements and the actual measurements of the holotype, small discrepencies in some aspects of the description, and the presence of a spermatheca. The spermatheca is not obvious in all specimens, its visibility is dependent on the orientation of the diverticulum in relation to the uterus, and to the condition of the uterine region after the animal has been fixed and processed to glycerine. The uterine region is poorly preserved in the holotype specimen and the diverticulum is not apparent, but in two of the paratypes it is clearly evident. The other two paratypes examined have the appearance of late fourth stage juveniles in which the spermatheca is not formed. For reasons of clarity, it is intended to lodge additional specimens with the original collection.

On receipt of the holotype slide, the specimen was located under the ringing material and had to be remounted to enable close examination. The reduction of the value of 'a', may therefore be accounted for by the fact that compression occurred during storage, but this does not explain the discrepencies for values of 'c', 'V', spear length, or body length, or the fact that 'c' and 'V' were outside the range given for paratypes.

4.4.A.IV Tylenchus rikus n.sp. (Figure 12 A-D)

Holotype female: L = 386μ ; a = 32.1; b = 4.5; c = 3.7; V = 57.2%; G_1 = 24.6%; G_2 = 2.3%.

Paratypes (n = 19): L = 375-425μ (398); a = 29.0-37.1 (33.9); b = 4.3-4.9 (4.6); c = 3.5-3.9 (3.7); V = 56.1-58.9% (57.7); G_1 = 16.7-25.8% (22.5); spear = 6-7μ . Males unknown.

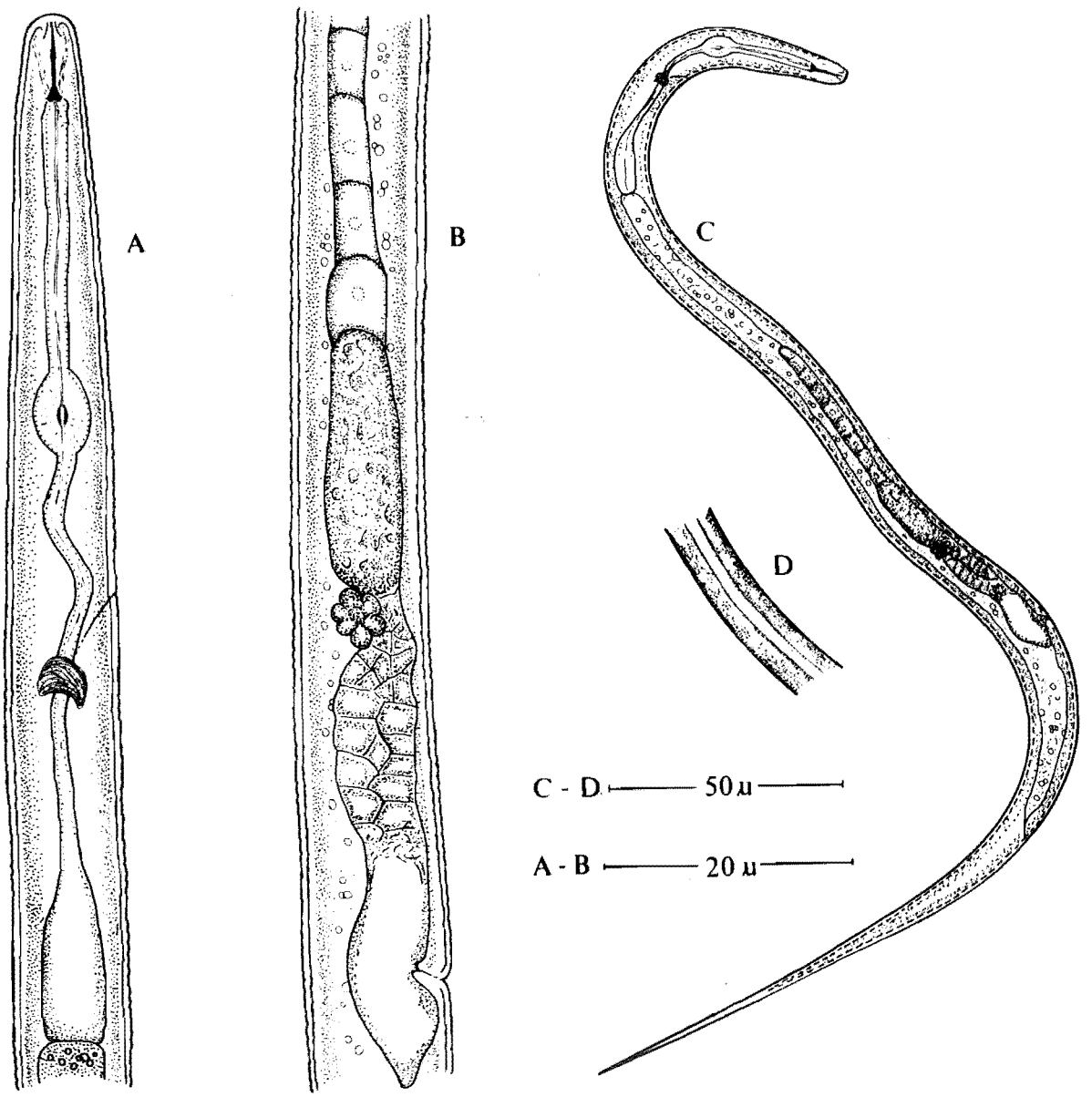
Body filiform, tapering anteriorly from about the level of the anterior extremity of the gonad and tapering markedly towards the posterior from the level of the vulva. Cuticle finely annulated; annules about 0.7μ wide at mid-body. Head narrower than body, not offset, with two fine annules. Tail with a long finely attenuated terminus, usually curved dorsally when relaxed by gentle heat (figure 12C).

Two lateral field incisures (Figure 12D); lateral field about 20% of the greatest body diameter, extends over about 60% of the body length. The incisures originate together at the level of the median oesophageal bulb and terminate about three anal-body-width behind the anus. Deirids not seen.

Stylet fine and slender, about 6-7μ long, difficult to see in specimens processed to glycerine. Anterior conical section about 42% of the total length, basal knobs small, delta shaped, about 1.3μ across. Dorsal oesophageal gland orifice about 0.5μ behind the spear base (Figure 12A).

Median oesophageal bulb oval, 6-7μ long and 4 -5μ wide, located at about 40% of the oesophageal length; valve plates and musculature weakly developed. Isthmus long and slender, frequently folded anterior to the nerve ring in fixed specimens. Nerve ring slightly posterior to the mid-point of the isthmus, excretory pore anterior to nerve ring.

FIGURE 12. *Tylenchus rikus* n.sp.
A) Oesophageal region. B) Female sex organs. C) Entire female.
D) Lateral field.


A
B
C
D
C - D 50 µ
A - B 20 µ

Vulva a transverse slit, slightly depressed. Vagina with thick walls, uterus consisting of a clear area about 10μ long anteriad, leading to a section of large columnar cells, usually two cells deep, about 20μ long with a spermatheca evident as a small convoluted diverticulum at the level of the oviduct (Figure 12B). The ovary is single, consists of 8-12 oocytes arranged in single file; post-uterine sac about 10μ long.

Type locality: From soil around roots of Festuca novae-zelandiae at Broken River (map reference N.Z.M.S. 1, Sheet S66); altitude, 720 m.s.l.

Diagnosis: Tylenchus rikus is distinguished by its small size; attentuated body with a long tail; fine cuticular annulations; small spear with delta shaped basal knobs; two lateral field incisures; and the presence of a small convoluted diverticulate spermatheca. It differs from most other small Tylenchus species by the presence of two lateral field incisures and the possession of a diverticulate spermatheca, but is close to Tylenchus helenae Szczygiel, 1969, Tylenchus parvus Siddiqui, 1963, and Tylenchus (Lelenchus) clarki Egunjobi, 1968, which are small species with two incisures in the lateral field. T. rikus differs from T. helenae by its smaller size (T. helenae, 0.43-0.48mm long), and presence of the diverticulate spermatheca (spermatheca not reported for T. helenae); from T. (A) parvus by its smaller size (T.(A) parvus 0.43-0.54mm long), finer cuticular striations (striae 1.8μ apart at mid-body of T. (A) parvus), the absence of lateral vulval membranes, and a more anteriorly located vulva (V = 61-66% in T. (A) parvus); from T. (L) clarki by its longer tail (c = 4.7-8.2 for T. (L) clarki), the presence of a post-uterine sac (absent in T. (L) clarki), and the diverticulate spermatheca (spermatheca figured as a tubular structure for T. (L) clarki).

The specific epithet 'rikus' is derived from the Maori word 'riki', meaning small.

4.4.A.V Tylenchus maiakus n.sp. (Figure 13 A-D)

Holotype female: L = 502μ ; a = 45.6; b = 5.8; c = 2.9; V = 50.2%; G_1 = 23.3%; G_2 = 1.2%; spear = 7.5μ .

Paratypes (n = 13 females): L = 435-530μ (498.6); a = 40.8-50.0 (46.2); b = 4.5-6.0 (5.4); c = 2.8-3.3 (3.1); V = 49.5-54.3% (51.5); G_1 = 16.6-24.0 (19.3); spear = 7.5-8.5μ .

Males unknown.

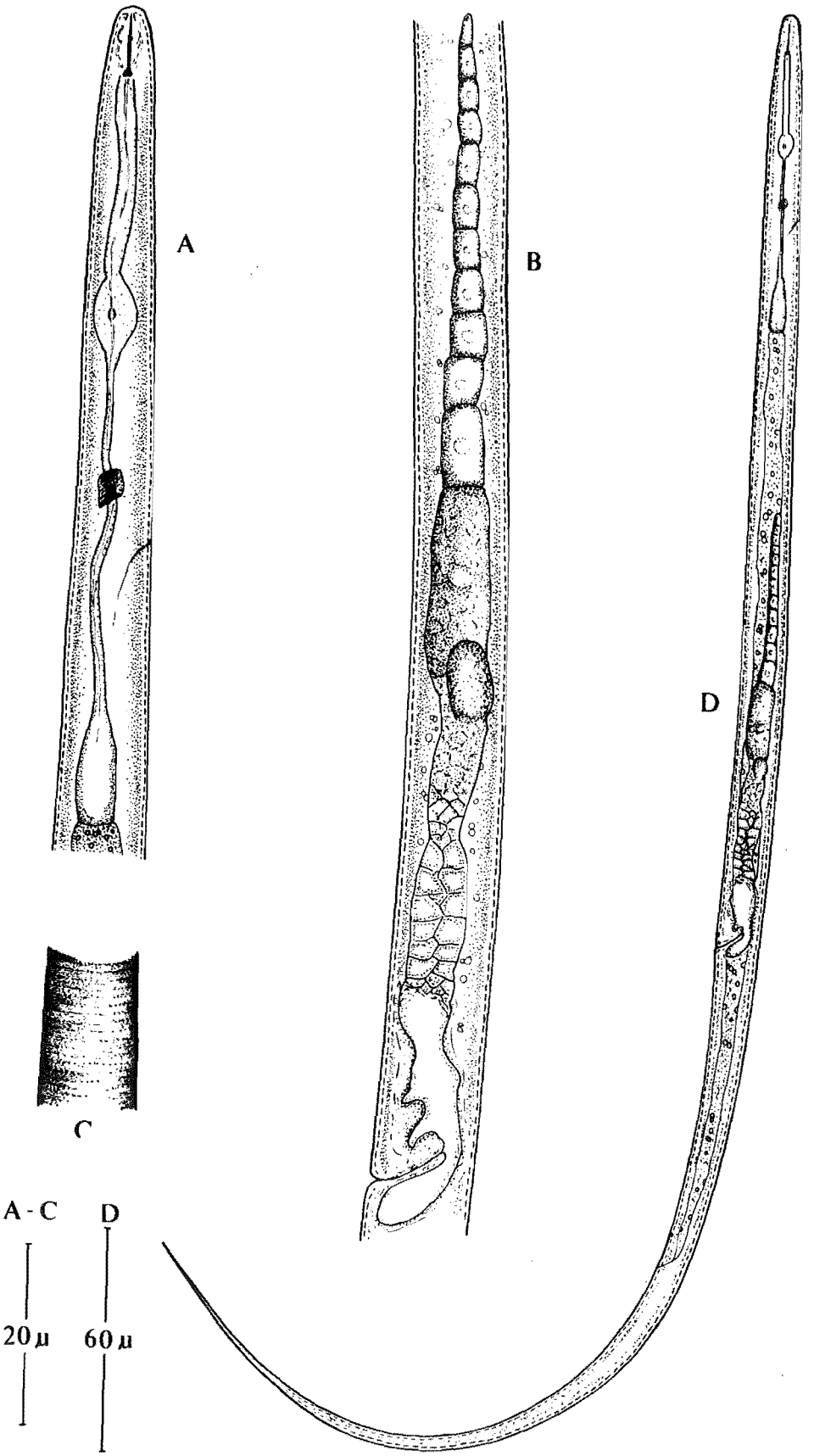
Females: Body filiform, tapering anteriorly from about the level of the oesophageal gland, and from the vulva posteriorly (Figure 13D). Cuticle appears smooth, but on close examination, extremely fine transverse striae, about 0.8μ apart may be distinguished (Figure 13C). Lateral fields and deirids not seen. Tail long (about 30% of the body length), with a finely attentuated terminus. Body usually lies straight when relaxed by gentle heat. Head bluntly rounded, about 4μ wide and 2μ high, not offset from the body.

Stylet fine and slender, about 8μ long, anterior conical section about 40% of the total length. Basal knobs present, small, about 1.5μ across, delta shaped (Figure 13A).

Procorpus of oesophagus 2-3μ in diameter, straight. Median oesophageal bulb located at about 40% of the oesophagus length, oval, 9-10μ long and 5-6μ wide, with centrally located valve plates, about 1.5μ long.

Isthmus long and slender, comprising about 35-40% of the length of the oesophagus, nerve ring located just anterior to the mid-point, at about 60% of the oesophageal length. Excretory pore posterior to the nerve ring, at about 70% of the oesophageal length, opening at the cuticle

FIGURE 13. *Tylenchus maiakus* n.sp.
A) Oesophageal region. B) Female sex organs. C) Cuticular sculpture. D) Entire female.


A
B
C
D
A - C
D
20 µ
60 µ

in a depression about 1.0μ wide. Terminal oesophageal bulb sacculiform, about 17μ long and 6μ wide, abutting with the intestine (Figure 13A).

Vulva a transverse slit about 5μ across, in a recessed position. Vagina with thickened walls, slopes anteriad, and in lateral view extends across 75% of the body width (Figure 13B). The vagina leads to a muscular section of the uterus about 10μ long anteriorly, which is continuous with a short (6-10μ long) post-uterine sac. A section of large cells, two to three deep and about 8-9 cells long extends for about 20μ anteriad from the muscular area. A short spermatheca is present, with an overlapping small, rounded, diverticulum at the junction with the uterus (Figure 13B). Ovary single, consisting of about 12 oocytes arranged in single file.

Type locality: From soil around roots of Festuca novae-zelandiae at Broken River (map reference N.Z.M.S. 1 Sheet S66); altitude, 720 m.s.l.

Diagnosis: Tylenchus maiakus is distinguished by its attenuated body with a long tail; bluntly rounded head; the lack of obvious cuticular annulations; its small spear; the oblique vagina; the location of the vulva about mid-body, and the presence of a diverticulum associated with the spermatheca. It is close to Tylenchus (Lelenchus) leptosoma de Man, 1880, Andrassy, 1964 but differs from that species by the smoothness of the cuticle (cuticle of T. (L) leptostoma finely annulated (Loof, 1961). The shape of the head (conoid in T. (L) leptostoma), the location of the excretory pore (at the beginning of the terminal oesophageal bulb in T. (L) leptostoma), and the presence of a diverticulate spermatheca.

The specific epithet 'maiakus' is derived from the Maori word 'maiaka',meaning thin.

4.4.B Biology

Despite the common occurrence of tylenchs in the rhizosphere, very little has been reported on their bionomics. Several workers have suggested that they probably feed on fungi or root hairs (e.g. Hirschmann, 1960; Seinhorst, 1961; Thorne, 1961), but until the present feeding trials (Chapter 3) were carried out the evidence indicated that they were root parasites. Cobb (1925) observed Tylenchus costatus de Man, 1921, in swellings of Paeonia officinalis roots, but did not demonstrate active parasitism. Khera and Zuckerman (1962) established populations of Tylenchus agricola de Man, 1884, on alfalfa callus tissue, and the same authors (Khera and Zuckerman, 1963), described the feeding of T. agricola and Tylenchus bryophilus Steiner, 1914, on seedling roots of alfalfa and dill (Anethum graveolens L.) in agar culture. Populations of Tylenchus emarginatus Cobb, 1893, were established on Picea mariana (Mill.) B.S.P., under greenhouse conditions by Sutherland and Keeble (1966). Later, Sutherland (1967a) showed that T. emarginatus reproduced on 11 out of 12 species of forest nursery seedlings in host range studies, and in the same year (Sutherland, 1967b), described the mode of parasitism of T. emarginatus on seedling roots of red spruce (Picea rubens Sarg.) and black spruce (P. mariana), in agar culture. Recently Gowen (1970), reported observations on the fecundity and longevity of T. emarginatus on sitka spruce (P. sitchensis (Bong.) Carr) seedlings at different temperatures and noted the plant parasitic habit of T. costatus.

4.4.B.I Aglenchus neozelandicus (Egunjobi, 1967)n.comb.

INTRODUCTION

A. neozelandicus is a component of the rhizosphere

fauna at Broken River and as was shown in Chapter 3, it is a plant feeding species. To develop an understanding of the role of A. neozelandicus in the biology of the soil, and to develop a background knowledge for the interpretation of its potential as a plant pathogen, detailed observations on feeding, host range, host attraction and life-history were made.

METHOD

Host range: In preliminary screening trials, a range of seedlings of pasture species were tested as potential nematode hosts under culture conditions. Seedlings of white clover, suckling clover, perennial ryegrass, crested dogstail, yorkshire fog, timothy, goosegrass, sweet vernal, browntop, cocksfoot, fescue tussock and chewing fescue grown asceptically in 1.0% water agar were inoculated with A. neozelandicus. Regular observations were made to establish whether or not feeding occurred. Subsequent host range trials were carried out under green-house conditions. Surface sterilised seeds of the pasture species listed above were sown in sterilised soil. Four weeks after sowing the seedlings were thinned to 10 per container, and three weeks later two pots for each plant species were inoculated with 30 surface sterilised A. neozelandicus. For reasons explained previously, fescue tussock seedlings were grown for 10 weeks before they were inoculated. Eight months after inoculation population levels in each of the pots were determined.

Penetration and feeding: Observations on penetration and feeding were made on nematodes parasitising seedling roots of perennial ryegrass, short rotation ryegrass and fescue tussock grown under asceptic conditions

in 1.0% water agar. Seedlings were germinated in water agar and when the radicle was about 0.5-1.0cm long, the Petri plate was inverted. With a normal geotropic response the roots grew downwards toward the surface of the medium. A cover-glass was placed over the root tip causing subsequent growth to continue in close contact to the glass. Surface sterilised nematodes were introduced and feeding could be observed at high magnifications by focussing through the thin cover-glass.

Life-history: Gravid adult A. neozelandicus obtained from lucerne tissue cultures were placed on roots of short rotation ryegrass seedlings grown under asceptic conditions and maintained in a growth room at 20°C. A continued supply of young roots was ensured by placing freshly germinated seeds on the medium at weekly intervals. It was sometimes necessary to pour cooled (45°C) 0.8% water onto the surface of the plates to prevent drying out, or when excessive contamination occurred through handling the plates, to transfer the animals to fresh cultures. Regular examinations of nematode development were made through the bottom of the plates using a compound microscope.

The effects of temperature on the time for egg development were investigated using fescue tussock seedlings parasitised by gravid A. neozelandicus. When at least 10 eggs had been laid, the cultures were incubated at 5°C, 10°C, 15°C, 20°C and 25°C. The number of eggs and their stage of development at the time of incubation was noted.

Host attraction: Surface sterilised short rotation ryegrass seed was germinated in 1.0% water agar. When the roots were about 2cm long, 20 surface sterilised A. neozelandicus were introduced at distances of approximately

3.0mm, 5.0mm, 10mm, and 20mm from them. The animals were individually pushed into the side of small wells about 5.0mm square, cut into the agar. The number of nematodes feeding within the root hair zone or on the main root was recorded six hours and 20 hours from the time the first nematode in each group was first placed on the agar. To record the pattern of movement of nematodes in the vicinity of seedling roots, individual animals were placed at measured distances from the radicles and their movement plotted by using a fine grid marked on the undersurface of the Petri plate.

Host selection: Surface sterilised seeds of sweet vernal, timothy, browntop, crested dogtail, yorkshire fog, clustered clover (Trifolium glomeratum L.), fescue tussock and short rotation ryegrass were germinated on 1.0% water agar. The Petri plates were tilted at an angle of 45° to promote root growth in a uniform direction. When the radicle was about 4.0mm long, each seedling was inoculated with 20 surface sterilised A. neozelandicus. Twelve hours later, a germinating seed of short rotation ryegrass was placed about 7.0mm from each of the inoculated seedlings. By placing the emerging radicle tip beneath the agar surface, and maintaining the angled position of the Petri plates, the ryegrass roots were encouraged to grow almost parallel to the roots of the parasitised seedlings. After three days, the cultures were examined to determine the location of the nematodes.

RESULTS

HOST RANGE: A. neozelandicus was observed feeding on all of the seedlings tested under culture conditions.

The suitability of the plants as hosts was confirmed by the establishment of populations of nematodes on the same range of species grown in soil under green-house conditions (Table 10).

PENETRATION AND FEEDING: A. neozelandicus was observed feeding ectoparasitically on root hairs (Figure 14A), epidermal root cells (Figure 14C), and occasionally on sloughed root cap cells (Figure 14B).

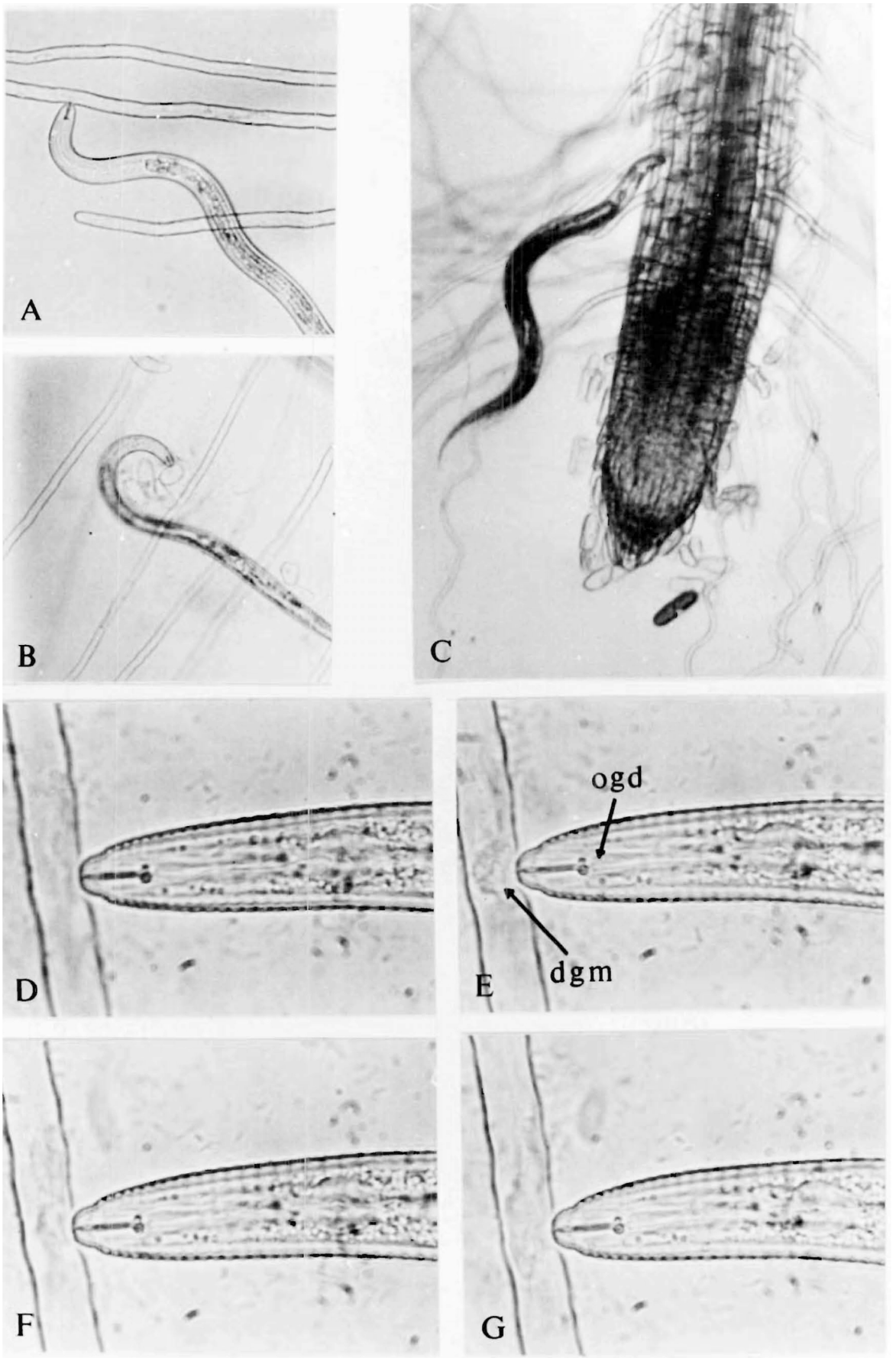
Penetration is achieved by repeated thrusts of the nematode's stylet against the cell wall. In some instances regular probes are made (120-150 per minute), in others irregular movement every two to five seconds is interspersed with four to five rapid thrusts directed against the cell wall. The time required to effect penetration ranges from about 10 seconds to about four minutes.

Penetration is followed by a short period during which no muscular activity is obvious. But during this period the region immediately behind the oesophageal gland duct (Figure 14E) at the base of the stylet becomes more refractive and appears to increase slightly in size. Feeding is initiated by simultaneous pulsation of the median oesophageal valve and the oesophageal gland. The forward and back movement of the posterior section of the oesophageal gland is associated with displacement of the oesophago-intestinal junction. About two minutes from the onset of feeding a cone of material appears within the parasitised cell around the orifice of the stylet (Figure 14 D-E). As feeding continues, the cone increases in size, sometimes filling the root hair protoplast (Figure 14 F-G). Cyclosis within the parasitised cell does not appear to be impeded during feeding; the cytoplasmic streaming continues, but flows around the dome of denser material often carrying away large portions with it.

TABLE 10: Populations of A. neozelandicus established on seedlings grown under green-house conditions in host range trials. Each pot contained 10 seedlings of one of the species listed below, and was harvested eight months after inoculation with 30 nematodes.

Plant species	Number of nematodes per ml of soil		
	Pot 1	Pot 2	Mean
White clover	5.2	12.1	8.6
Suckling clover	0.0	4.0	2.0
Perennial ryegrass	29.5	13.3	21.4
Crested dogstail	8.5	15.8	12.1
Yorkshire fog	3.1	19.2	11.1
Timothy	19.1	16.7	17.9
Goosegrass	9.4	14.2	11.8
Sweet vernal	16.3	12.8	14.5
Browntop	18.1	7.8	12.9
Cocksfoot	15.1	24.0	19.5
Fescue tussock	8.6	2.5	5.5
Chewings fescue	9.6	8.1	8.8

FIGURE 14. Feeding of Aglenchus neozelandicus. on ryegrass seedlings growing in water agar.
A) Feeding on root hairs. B) Feeding on cast root-cap cells. C) Feeding on epidermal cells. D-G) Feeding on a root hair showing build-up of granular material about the stylet orifice (ogd= oesophageal gland duct; dgm= dome of granular material).


A
B
C
D
ogd
dgm
E
F
G

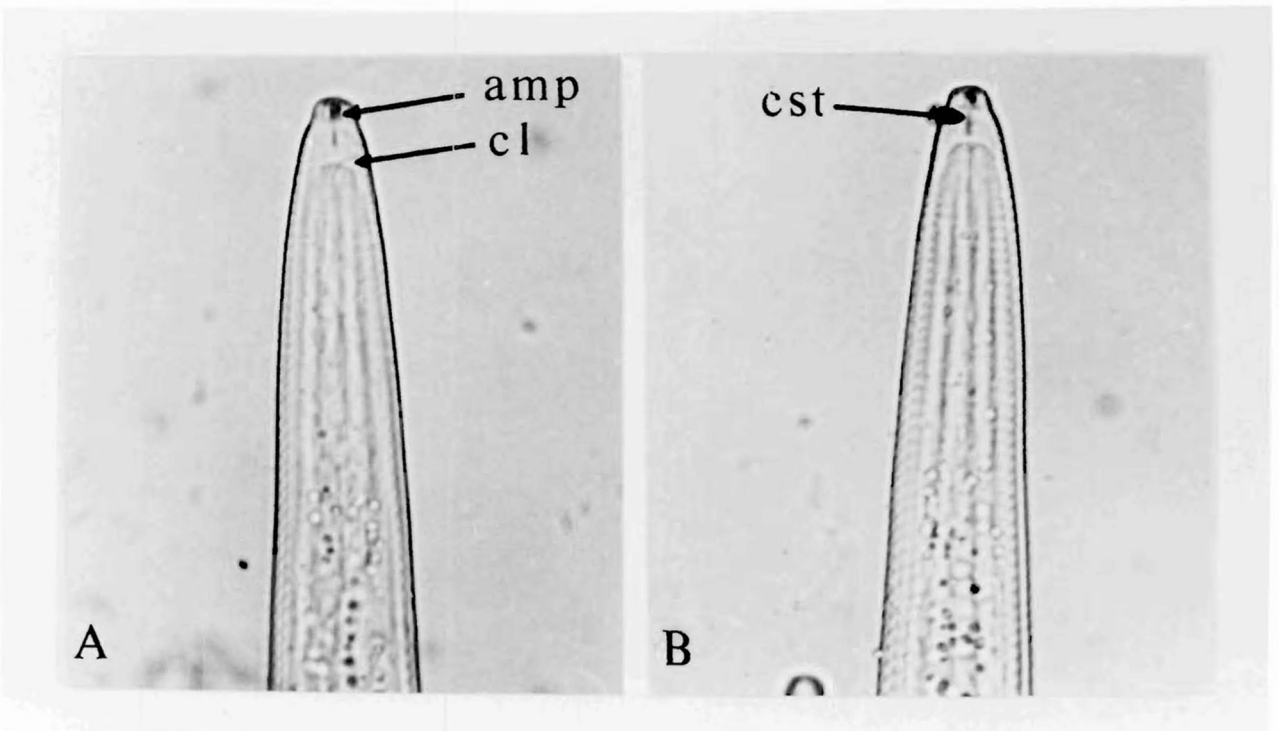
The feeding nematode frequently alters the angle of the stylet without shifting its head position. Feeding periods of 30 seconds to three hours have been observed, but generally last about 15-20 minutes. Stylet movement and pulsation of the oesophageal valve may occur a few seconds after the stylet is withdrawn from the host.

LIFE-HISTORY: Adult A. neozelandicus usually begin laying within 24 hours of inoculation onto seedling roots. Eggs measure 53-63μ x 14-19μ . The first moult occurs within the egg five to six days after laying. Second stage juveniles hatch after six to seven days and most begin feeding within 24 hours. Feeding is necessary for further development. Juveniles undergo three additional moults before reaching maturity. During a moult, the posterior portion of the stylet becomes faint, and the anterior portion is shed with the cuticle. As the cuticle is separated in the head region, two faint lines which appear to link the cast amphidial pouch with the new lips can be seen (Figure 15A-B). The moult of the pre-adult juvenile takes 35-45 hours to complete. Mature females begin laying two to three days after emerging. The generation time (egg to egg), for nematodes on short rotation ryegrass seedlings in 1.0% water agar at 18-20^{o}C was 27-35 days.

The duration of each stage may be summarised as follows:

Egg stage .....6 - 7 days;
Second stage juveniles.....5 - 7 days;
Third stage juveniles.....6 - 8 days;
Fourth stage juveniles.....8 -10 days;
Pre-oviposition period of adult...2 - 3 days.

FIGURE 15. Moulting Aglenchus neozelandicus.
A) Early moult stage-stylet not visible, connecting link between amphid pouches and lips can bee seen (amp= amphid pouch; cl= connecting link).
B) Late moult stage - stylet visible, cuticle annulation distinct, no apparent link between lips and amphid pouches (cst= cast spear tip).


amp
cl
A
cst
B

Dimensions of deveopment stages and adults:

Second stage juveniles (n = 10): L = 201-246μ (219); a = 20.9-25.8 (23.7); b = 3.0-3.7 (3.3); c = 3.6-3.9 (3.8); stylet = 7.0-8.0μ (7.7).

Third stage juveniles (n = 10): L = 271-355μ (310); a = 25.16-28.91 (26.4); b = 3.98-4.52 (4.3); c = 4.1-4.9 (4.4); stylet = 8.5-9.5μ (9.2).

Fourth stage juveniles (n = 10): L = 322-451μ (389); a = 24.2-27.5 (25.7); b = 4.5-5.1 (4.8); c = 4.3-5.2 (4.7); stylet = 9.5-11.0μ (10.1).

Adults females (n = 16): L = 44-505μ (468); a = 24.4-34.1 (28.5); b = 5.3-6.1 (5.7); c = 4.8-5.8 (5.2); G_1 = 21.8-32.0% (26.4); V = 62.6-70.0% (64.9); stylet = 10.0-12.0μ (11.2).

Fecundity: Eggs were laid at about 16 hour intervals for the first three to four days, increasing to about one every 10 hours by the seventh day. Adult females survived for up to 60 days at 18-20°C, but few continued to produce eggs later than 35 days after maturing. Three females which survived or were not lost over a period of six weeks laid 62, 95 and 109 eggs respectively.

Effect of temperature on egg hatch: None of the eggs incubated at 5°C hatched after 30 days. At 10°C two eggs from 12 hatched after 25 and 26 days respectively; in the remainder, development appeared to be arrested at the tadpole stage. The development time was 13-17 days at 15°C; 6-7 days at 20°C, and 5-7 days at 25°C.

ORIENTATION: The results presented in Table 11 demonstrate the positive orientation of A. neozelandicus toward seedling roots in water agar. Additional evidence is provided by the plots of movement of individual nematodes placed in close proximity to the seedling roots (Figure 16). For most animals a period of limited activity occurs before a

FIGURE 16. Migration patterns of nematodes towards ryegrass seedling roots in water agar.

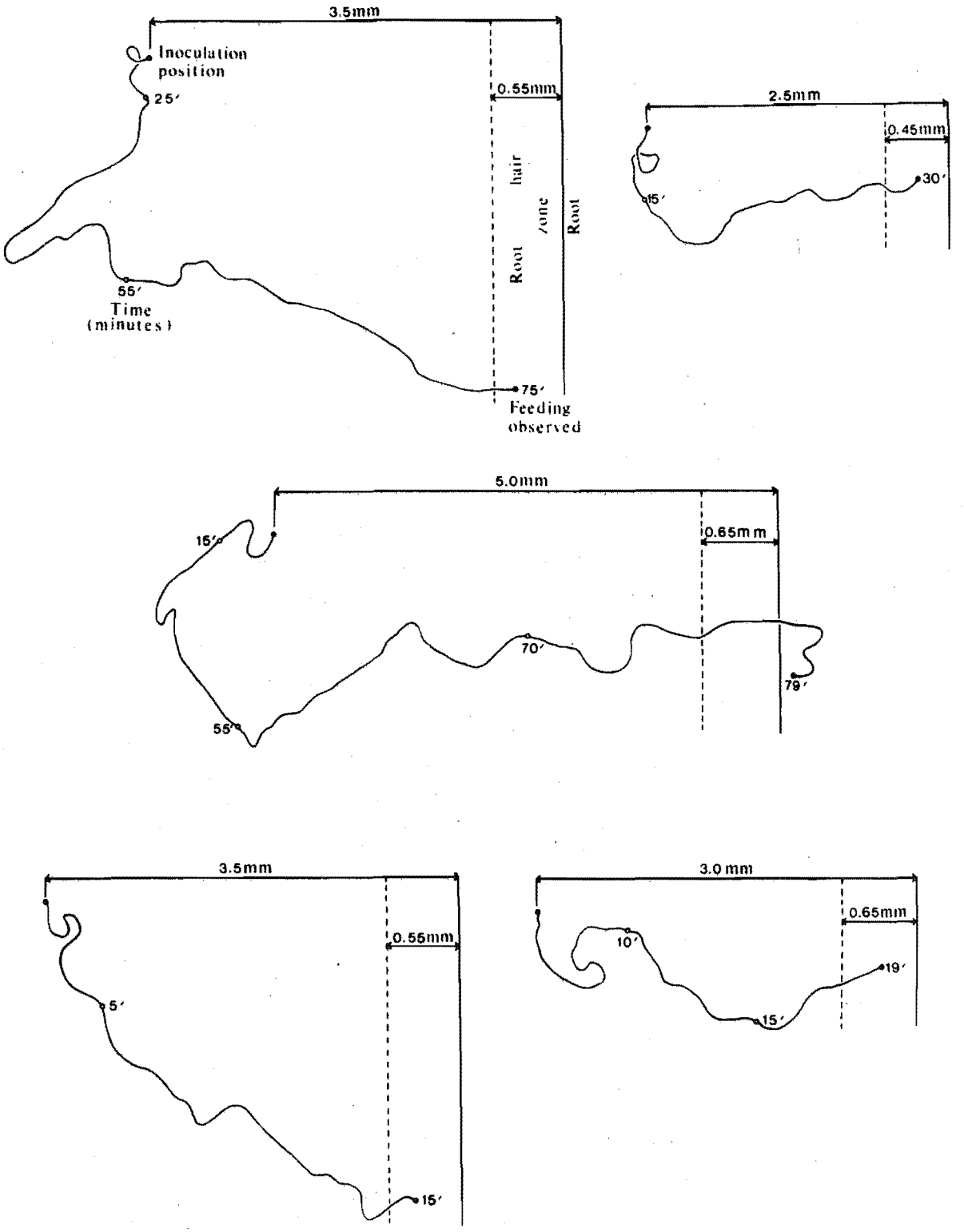

3.5mm
Inoculation position
25′
0.55mm
Root hair zone
Root
55′
Time (minutes)
75′
Feeding observed
2.5mm
0.45mm
15′
30′
5.0mm
15′
0.65mm
70′
79′
55′
3.5mm
0.55mm
5′
15′
3.0mm
0.65mm
10′
19′
15′

TABLE 11: Migration of A.neozelandicus to short rotation ryegrass roots with time.

Distance from roots mm	Numbers of A. neozelandicus feeding on roots after		
	2 hours	6 hours	20 hours
3	6	12	17
5	10	17	18
10	2	8	12
20	0	5	15

TABLE 12: Migration of A. neozelandicus between seedlings of different plant species grown in 1.0% water agar.

Plant species combination			Nematode number (after three days)	
			Replicate 1	Replicate 2
Clustered clover	x	short rotation ryegrass	6 x 11	6 x 10
Yorkshire fog	x	"	9 x 8	6 x 6
Sweet vernal	x	"	12 x 7	9 x 7
Timothy	x	"	10 x 6	7 x 8
Crested dogstail	x	"	13 x 6	11 x 8
Fescue tussock	x	"	9 x 4	9 x 7
Browntop	x	"	6 x 11	7 x 11
S. R. Ryegrass	x	"	10 x 8	11 x 5

definite response to the position of the roots is evident. As the nematode-root distance is reduced, movement becomes more positively directed toward the root.

HOST SELECTION: Migration of A. neozelandicus between feeding periods is indicated by dispersal of nematodes between two seedlings growing close together (Table 12), but there is no absolute host preference shown by total migration to any one plant species. The root hair zone of some species (e.g. browntop and clustered clover) is small relative to other species, and in particular, to the root hair zone of short rotation ryegrass. The probability of migrating nematodes contacting the roots of ryegrass seedlings in combination with such species is proportionately greater, that is, providing there is no host specific attraction factor. This probably accounts for the apparent shift of more nematodes from browntop and clustered clover to short rotation ryegrass.

DISCUSSION

A. neozelandicus exhibited a wide host range on seedlings grown in water agar and on plants grown under green-house conditions. Further, there was no evidence of differential attraction between seedlings of different species grown in water agar. Hence it is suggested that the distribution pattern of plant species is unlikely to have a major influence on the distribution pattern of the nematode. However, with such a wide host range, it is apparent that detrimental effects of nematode feeding on roots on plant growth may have widespread consequences in a close sward of pasture plants.

The pattern of penetration and feeding by A. neozelandicus is similar to that described for several ectoparasitic species (e.g. McElroy and Van Gundy, 1968; Dropkin, 1969), and follows closely the pattern of Tylenchus emarginatus observed by Sutherland (1967b). Sutherland (1967b) considered that the dome of granular material which formed around the nematode stylet during feeding, resulted from movement of material toward the stylet orifice during periods of pulsation of the median oesophageal bulb. Movement of granular material toward the stylet orifice of A. neozelandicus was not observed in the present trials. Indeed granular material was observed streaming around the periphery of the dome during cyclosis of the cell contents. The increase in size of the 'bulb' associated with the dorsal oesophageal gland duct indicates that the nematode produces a substance in the oesophageal glands which is moved down to the duct behind the stylet during feeding. Dropkin (1969) suggests that such a system is designed to deliver secretions to the plant cell via the stylet and notes the possible implication of extra-oral digestion. From observations on the feeding of A. neozelandicus it is suggested that the localised dome of material around the stylet tip during feeding is either a substance produced by the nematode and introduced into the host, or a zone of physiologically modified host cytoplasm induced by secretion of material by the nematode. The dense appearance of the dome, and the fact that portions of it retain their shape when dislodged and carried away in the cytoplasmic currents suggests gel-like properties. Doncaster (1966) noted that during feeding of Ditylenchus destructor Thorne, 1945, and D. myceliophagus J. B. Goodey, 1958, on Botrytis cinerea Pers. ex. Fr., endoplasm stopped streaming soon after the stylets entered, suggesting changes in the properties of the protoplasm which favoured ingestion

by the nematodes. Doncaster (1966) considered that changes in the colloidal properties or gelation possibly occurred which would minimise the risk of solid or viscous ingredients blocking the stylet during ingestion. In addition, it is here suggested that in an unmodified cell, the rapid flow of cytoplasm over the small aperture of an intruding stylet could possibly act in the manner of a venturi, creating a negative pressure which the nematode would have to overcome. An increase in the viscosity of the cytoplasm around the stylet orifice and thence minimisation of movement may facilitate ingestion by the nematode. Khera and Zuckerman (1963) have also noted movement of the stylet and pumping of the median oesophageal bulb after removal of the stylet of T. bryophilus from root cells of alfalfa and dill. In A. neozelandicus this may be involved in clearing food substances from the oesophagus, or glandular secretions from the stylet.

The time required for the completion of the life-cycle of A. neozelandicus is similar to that recorded for most plant parasitic nematodes; Wallace (1963), cites a period in the order of 20-40 days as common. Gowen (1970) recorded a generation time of five to six days for species T. emarginatus at 25°C, but Sutherland (1967b) found that eggs of this species required five days to hatch at 23-26°C. A reduction of incubation temperature of A. neozelandicus eggs from 20°C to 15°C increased the development time from six to seven days to 13-17 days; few eggs hatched at 10°C, and none at 5°C. It is suggested that the cardinal minimum temperature for reproduction is probably about 10°C.

A. neozelandicus is attracted to seedling roots growing in 1.0% water agar. Since Linford (1939) observed that root-knot nematode juveniles congregate around roots, there have been a number of studies confirming the attraction of nematodes to plant roots (see Wallace, 1963; Klingler, 1965).

Several mechanisms have been postulated to explain the phenomenon. Attraction to CO_2 released by respiring roots was proposed by Klingler (1961). Nichols, Johnson and Viglierchio (1961) produced experimental evidence to support this hypothesis. Bird (1959) showed that certain reducing agents attracted nematodes and proposed a theory of attraction to areas of low redox potential. In the same year Kühn (1959) suggested that nematodes meet roots by chance and enter them or alter their movement under the influence of substances emitted by the roots; while they are under the influence of these substances they do not leave the rhizosphere, and so accumulate there. Although the arrival of nematodes at roots by random movement seems inevitable, the weight of opinion indicates that attraction over some distance does occur, and that root exudates are involved (e.g. Lownsberry and Viglierchio, 1960, 1961; Peacock, 1959, 1961; Bird, 1960; Blake, 1962; Van Gundy and Rackham, 1961; Klingler, 1965). The production of exudates by plant roots is well established and the composition of the exudates produced is complex (e.g. Katznelson, Rouatt and Payne, 1955; Parkinson, 1955; Sulochana 1962 a, 1962b; Vancura, 1964; Rovira, 1965a; MacRae and Castro, 1966). In a pasture soil the top 7.0-10.0cm is interlaced with plant roots. Under these conditions it is difficult to envisage the maintainance of diffusion gradients of root exudates in the soil apart from an extremely localised zone in the immediate rhizosphere. Although attraction of A. neozelandicus to plant roots may occur at a distance of 20mm in a sterile environment, movement of this order seems unlikely to occur in the soil. The effects of root exudates may be of significance over small distances however, and they are probably involved in the retention of a position close to the root during migratory activity between feeding periods as suggested by Kühn (1959).

4.4.B.II *Tylenchus rikus* n. sp.

INTRODUCTION

T. rikus is a fungus feeding nematode (see Chapter 3). In view of the relative ease with which fungus feeding nematodes may be cultured in the laboratory, and paucity of information on the biology of *Tylenchus* species, studies on the biology of *T. rikus* were undertaken.

METHOD

Host range: Five millimetre diameter discs of the fungi *Rhizoctonia solani* Kuhn, *Ulocladium atrum* Preuss, *Fusarium oxysporum* (Schlecht. ex. Fr.), *Sclerotinia sclerotiorum* (Lib.) de Bary, *Alternaria brassicicola* (Schw.) Wiltshire, *Stemphylium botryosum* Wallr., *Trichothecium roseum* (Pers.) Link ex. S. F. Gray, *Botrytis cinerea* Pers. ex. Fr., *Zygorrhynchus moelleri* Vuill., *Gliocladium roseum* Bain, *Chaetomium globosum* Kunze ex. Fr., *Phoma* sp., *Alternaria tenuis* Auct., *Ascochyta* sp., *Pythium* sp., *Mortierella alpina* Peyr., and *Aspergillus niger* van Tiegh., were placed in the centre of 9 cm diameter Petri plates containing one-half strength cornmeal agar, and incubated at 20°C for five days before the cultures were inoculated with *T. rikus*. Two plates for each fungus species were inoculated with 0.5ml of a suspension of surface sterilised *T. rikus*. A fungus was considered to be favourable if developing eggs and active nematodes of all stages were observed after a further three weeks incubation at 20°C. The level of inoculum ranged from 28 to 106 nematodes in five 0.5ml samples counted. No attempt was made to assess 'suitability between hosts'.

Feeding and life-history: Observations on feeding and life-history were made on animals cultured on U. atrum on cornmeal agar or 1.0% water agar in glass ring observation chambers and 9.0cm diameter Petri plates.

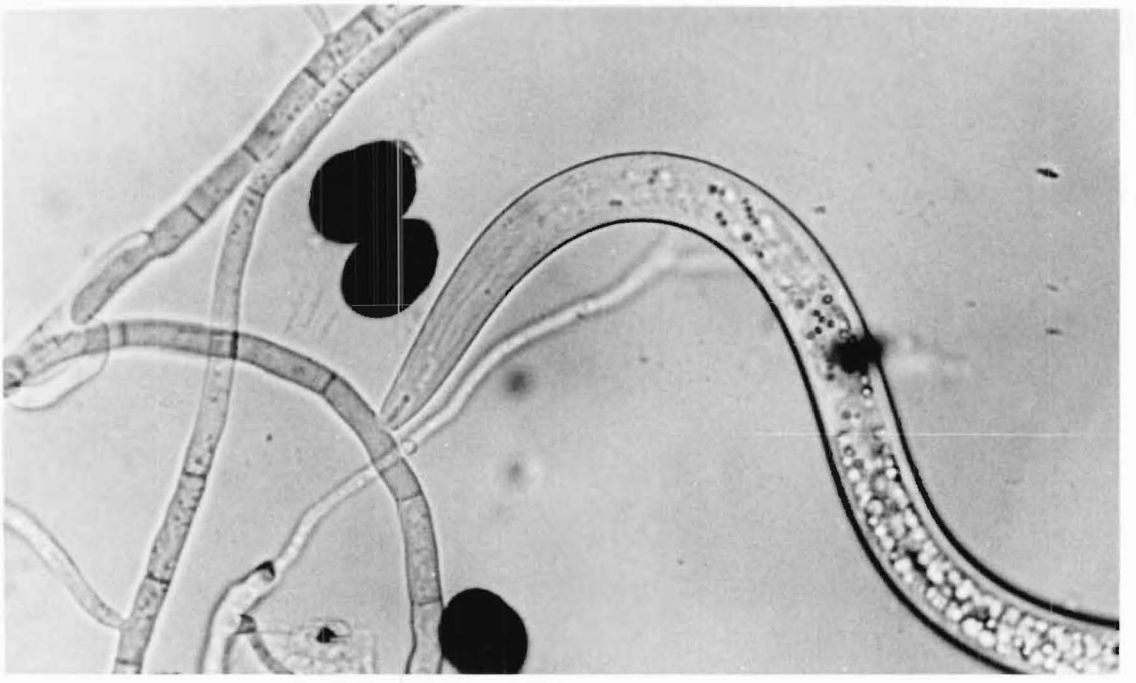
RESULTS

FEEDING: On contact with a fungus hypha, T. rikus assumes a position characterised by curvature of the cephalic region (Figure 17). Rythmic protrusion of the stylet tip against the hyphal cell wall follows (about one to two probes per second), until penetration occurs about 90 seconds later (range, 20-180 seconds). A rapid twitching movement of the median oesophageal valve begins, accompanied by pulsation of the oesophageal gland. The movements continue for about two minutes during which a faint hyaline globule appears behind the dorsal oesophageal gland duct. The pattern of movement of the median oesophageal gland changes from a lateral vibratory movement of the closed valve plates to a rythmic opening and closing of the plates as feeding begins. Pulsation of the oesophageal gland lobe continues. Lateral twitching movements of the posterior section of the lobe are accompanied by short forward and back movements of the anterior region. The hyaline globule at the oesophageal gland orifice reduces in size during feeding, but no movement of material to, or from, the stylet orifice was observed, nor was there evidence of modification of the host protoplasm. Feeding periods of five to 30 minutes were common.

HOST RANGE: Populations of T. rikus established on all of the fungi tested.

LIFE-HISTORY: Development within the egg: Eggs measure

FIGURE 17. *Tylenchus rikus* feeding on a hypha of *Ulocladium atrum*.



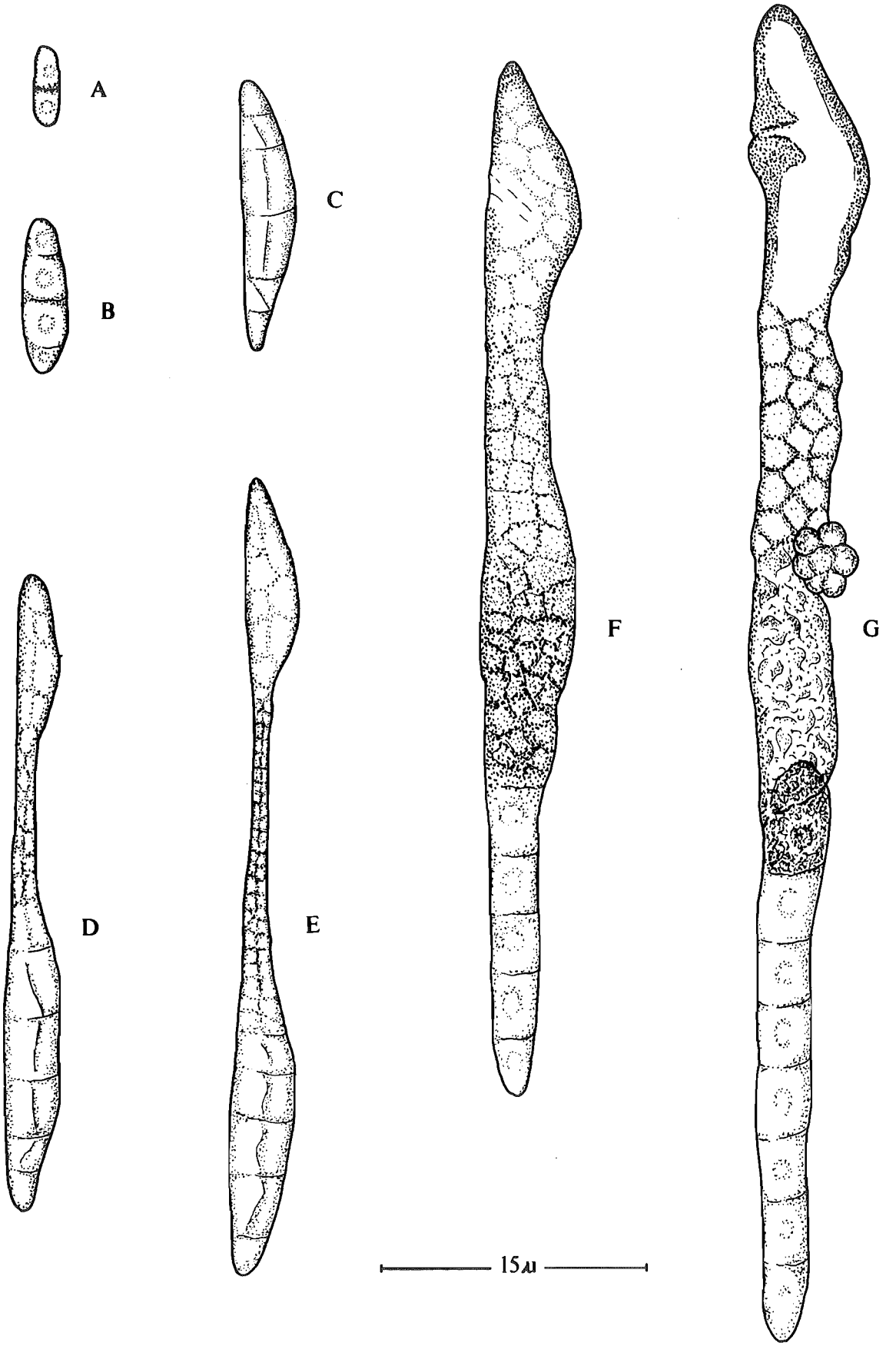
51.2-56.1μ (52.8) x 12.2-14.8μ(13.4) when laid (20 measured). They are elongate, bluntly rounded, often slightly curved, with a blunt micropyle-like scar about 1.0μ high and 2.0μ wide marking the end which is last to pass through the vulva (Figure 19D). The egg protoplasm is less dense at this end. The first cleavage occurs after 10-15 hours resulting in two cells of slightly unequal size. After about 20 hours the larger cell divides and division of the smaller cell follows 1-2 hours later. Movement of the embryo begins after about 55 hours. The embryonic nematode is well formed and active after about four days at which time it enters a period of relative inactivity lasting up to 20 hours. During this stage the stylet is differentiated and evidence of cuticle separation in the head region was noted but the actual moult was not observed.

Hatching: Five to six days from laying, movement of the juvenile becomes continuous and periods of stylet probing directed against the cell wall occur. The intensity of movement increases over the next few hours and pressure is applied to the poles of the egg by curvature of the cephalic region of the nematode. A few hours before hatching the egg increases in size and elasticity of the shell is evidenced by distortion as the juvenile continues to flex its body against the wall. At 20^{o}C hatching occurs five to six days from laying.

Post hatch: Measurements of second stage juveniles (n = 10): L = 195-250μ (217); a = 33.3-38.2 (33.7); b = 2.8-3.1 (2.9); c = 3.6-3.8 (two animals only); stylet = 5.5-6.5μ (6.0).

The genital primordium is 4.0-6.0μ long, at about 50% of the body length, composed of two cells (Figure 18A). The cuticle is finely striated and two lateral field incisures are just visible. Second stage juveniles feed for

FIGURE 18. Gonad development in Tylenchus rikus. A) Early post hatch stage. B) First moult stage. C-E) Third juvenile stage. F) Fourth juvenile stage. G) Late pre-adult moulting stage.


A
B
C
D
E
F
G
15 µ

three to five days before entering the second moult. Moulting second stage juveniles were 240-280μ long (10 observations) with a genital primordium consisting of four cells (Figure 18B).

Third stage: Measurements (n= 10): L = 270-340μ (314); a = 33.5-42.0 (36.3); b = 3.3-4.6 (3.8); c = 3.5-3.8 (3.7); stylet = 5.5-6.5μ (6.0).

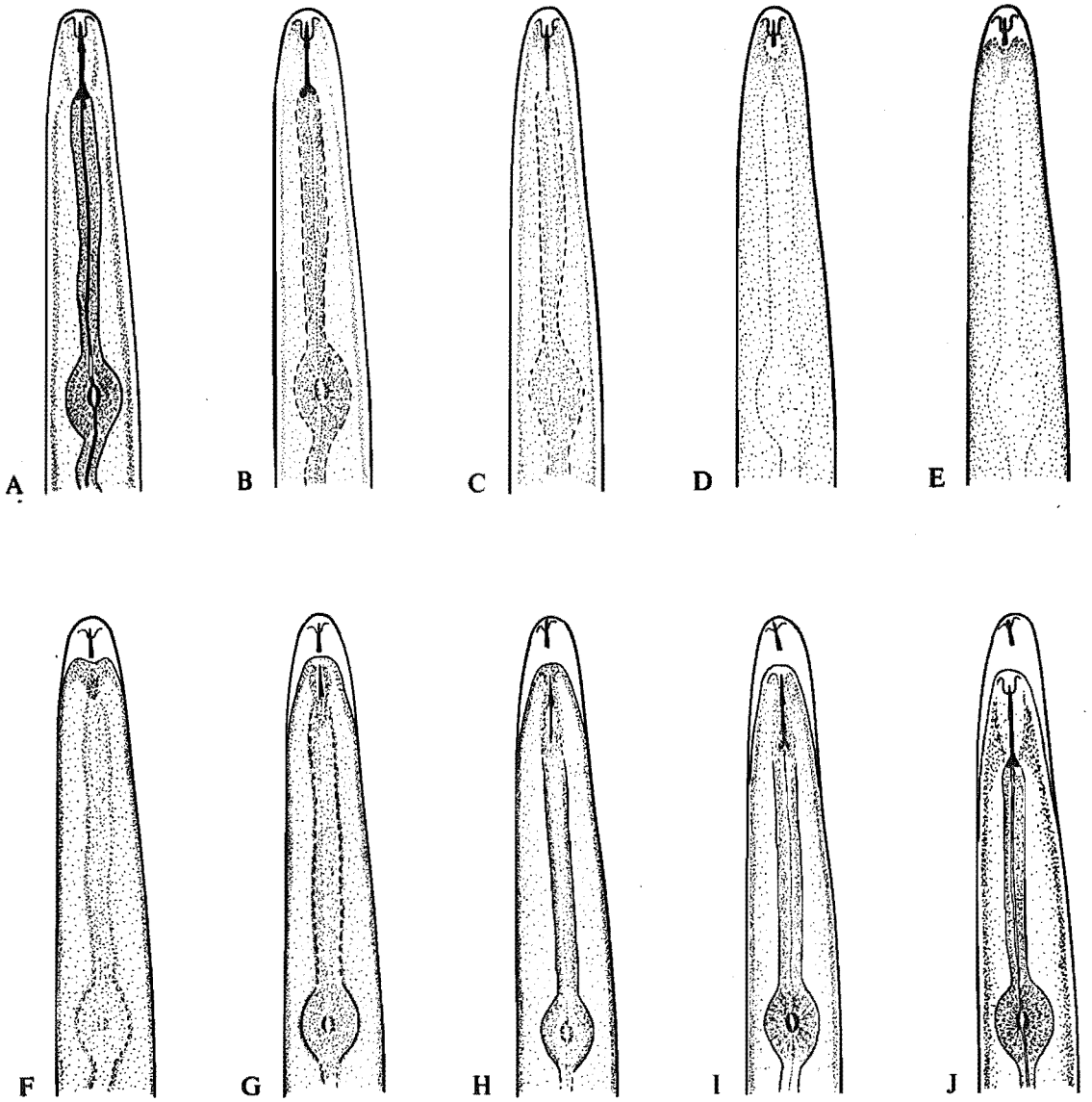
During the third stage the genital primordium increases in length from about 15μ to 40-50μ (Figure 18C - E). The individual somatic cells become smaller in size and their number increases; the germinal section is about 20μ long. The third moult occurs eight to nine days after the second. Moulting juveniles were 320-340 long (10 observations).

Pre-adult: Measurements of fourth stage juveniles; (n = 10): L = 335-390μ (365); a = 3.14-39.1 (34.3); b = 3.4-4.5 (4.0); c = 3.5-3.9 (3.8); stylet = 6.0-7.0μ (6.5).

In the fourth stage juvenile the vaginal primordium becomes more obvious, the region of the uteri and ovaries are differentiated, and the gonad extends in length anteriad and posteriad (Figure 18F). The final moult is entered eight to ten days after the third.

Pre-adult moult: The formation of a new stylet and cuticle replacement is similar in all post-hatch moults. Prior to moulting a nematode becomes sluggish, finally assuming a straight posture punctuated with occasional slow lateral movements of the head. The body contents become darker, with a granular appearance. Refractivity of the lumen of the oesophagus, valve plates of the median bulb, and the stylet decreases (Figure 19 A-C). About five hours after the beginning of the moult, only the conical apex of the stylet is visible (Figure 19D). A flask shaped hyaline area appears around the stylet apex (Figure

FIGURE 19. Pattern of moulting in the oesophageal region of Tylenchus rikus.
A-C) Refractivity of the stylet and oesophageal musculature decreases.
D) Appearance of a flask shaped hyaline area appears around the stylet orifice.
E) Separation of lips. F) Retraction of head and evidence of stylet formation.
G) Stylet apex formed. H-I) Stylet formed, oesophageal musculature evident. J) Basal knobs of stylet and oesophageal valve plates are formed.


A
B
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J

19D), and a new lip region begins to differentiate just behind the cephalic framework. Separation of the cuticle is distinct after about 10 hours (Figure 19E). The head slowly flattens and retracts from the old cuticle (Figure 19F). A granular area appears behind the lips (Figure 19F), as a new stylet apex becomes apparent (Figure 19G). At this stage, the excretory pore, oesophageal gland lobe and median oesophageal valve are visible, but the valve plates are still faint. A fine outline demarcating the vagina is visible. The hyaline cavity in the lip region disappears as sclerotisation of the stylet apex proceeds posteriad and the shaft begins to form (Figure 19H-I). Retraction of the head continues leaving the moulted stylet tip attached to the old cuticle. The developing vulva and vagina are apparent as an umbrella-like structure at this stage. After about 28 hours the new spear shaft is evident (Figure 19I) and about five hours later the basal knobs are completely formed (Figure 19I). Thirty-five hours from the beginning of the moult movement of the nematode is frequent, the vulva and the stylet are defined, and the median oesophageal valve plates are apparent (Figure 19J). The intensity of movement increases over the next five to six hours, lateral twitching of the head begins, and stylet probing associated with pulsation of the median oesophageal valve plates occurs. Contractile twitching movements extend to the rest of the body and separation of the two cuticles is effected. Cuticular striations on the new cuticle are marked. Forward and back movement interspersed with rotation in the longitudinal plane continues for about four hours. Body length appears to increase. Toward the end of the moult the nematode begins to apply pressure to the cast cuticle by arching the head region and pushing its lips and stylet against the wall. The moult is completed after about 48

hours,when the pre-adult cuticle ruptures just posterior to the lip region.

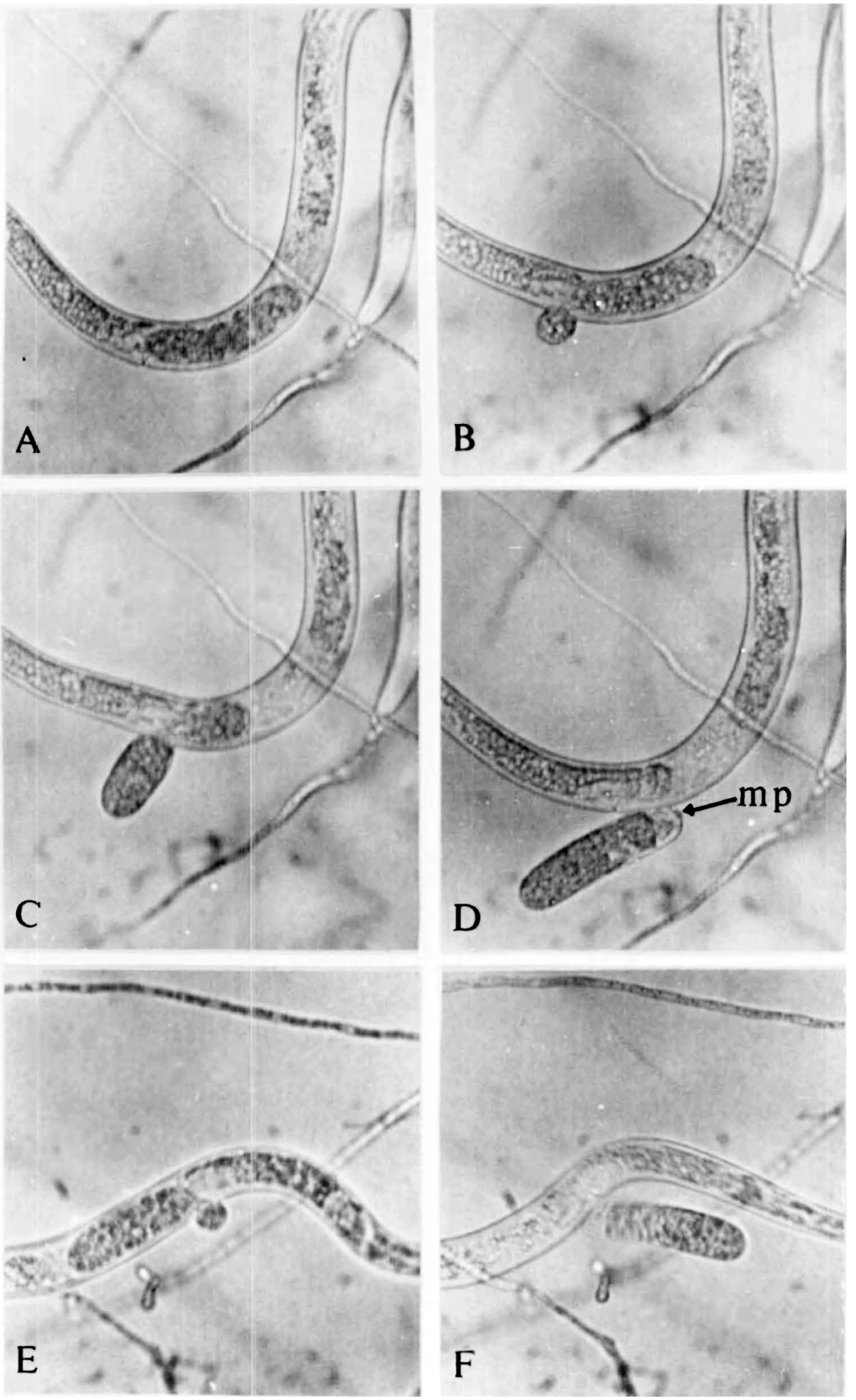
During the moult division of the cells of the gonad continues; oocytes are formed, the uterine wall cells take on their characteristic columnar appearance, and the convoluted diverticulate spermatheca is formed (Figure 18G).

Adult: Measurements (n = 19): L = 375-425 μ (398); a = 29.0-37.1 (33.9); b = 4.3-4.9 (4.6); c = 3.5-3.9 (3.7); V = 56.1-58.9% (57.7); G_1 = 16.7-25.8% (22.5); stylet = 6.0-7.0 μ .

Feeding occurs about 10 hours after the completion of the moult. The first egg is laid approximately 24 hours after its completion.

Egg laying: The movement of the egg in the uterus is slow. The time elapsed between movement of the granular oocyte through the constriction at the oviduct to laying of the egg ranges from two to six hours. Compaction of the developing egg occurs just posterior to the oviduct and is followed by evidence of shell deposition as slow movement of the egg down the uterine tract proceeds. Contraction of muscles associated with the post-vulval sac and vagina, aided by occasional flexing of the nematode body appear to control egg movement. The egg is finally forced into a position over the vulva and frequently extends into the post-uterine sac. Muscle movement is almost continuous, contractions sometimes lasting several seconds, causing displacement or partial opening of the sclerotised plates supporting the vulva. The egg is forced through the distended lips of the vulva with, or without, the aid of body flexures. It 'flows' through the narrow constriction and reforms the bluntly rounded elongate shape in the medium. Figure 20 illustrates both 'passive' and 'active' laying behaviour. In Figure 20A - D feeding

FIGURE 20. Laying behaviour of Tylenchus rikus. A-D) Feeding throughout laying with no marked body movement, (A-D, 11 seconds; mp= micropyle-like pore). E-F) Arching of body and flicking of tail during laying.


A
B
C
D
mp
E
F

continues throughout laying and no evidence of body movement can be seen; in Figure 20E-G arching of the body and tail movement accompanies deposition of egg. Eggs are deposited in an unsegmented condition. Females were observed to lay one to four eggs per day, but the total number of eggs laid by any one female was not determined.

Generation time: The generation time (egg to egg) on U. atrum at 20°C was 30-35 days.

Effect of temperature on egg hatch: No eggs incubated at 5°C hatched after 30 days; two eggs from eight hatched after 22 days at 10°C and an additional one after 28 days; development and hatching required nine to 11 days at 15°C (11 eggs); five to six days 20°C (10 eggs), and four to five and one-half days at 25°C (9 eggs).

DISCUSSION

The immediate post-penetration activity of T. rikus is typical of the post-penetration phase which precedes ingestion in many Tylenchoid nematodes (Dropkin, 1969). However feeding of T. rikus induced no obvious changes to the host cell protoplasm during feeding, and further, no cell changes were apparent on completion of the feeding period. If the swelling of the dorsal oesophageal gland duct can be taken as indicative of secretory activity of the dorsal gland, then it seems that the substance produced has no marked adverse on subsequent host growth. Thus with the wide host range shown for the nematode, T. rikus is well adapted to a mycophagus habit.

The occurrence of the first juvenile moult within the egg has been reported for many nematodes (e.g. Van Weerdt, 1960; Hirschmann, 1962; Clark, S.A., 1967). Similarly, a marked increase in shell flexibility prior to hatching has been noted in many species. Wilson (1958) suggested that the

pre-hatch activity of the juvenile assisted in the emulsification of the inner lipoid layer of the shell which promoted the uptake of water by osmosis and increased the pressure within the egg. With the additional effect of proteolytic enzymes secreted by the nematode the shell was ultimately ruptured. Increased movement of T. rikus prior to hatch may serve a similar function.

The genital primordium of second stage juveniles has two germinal nuclei. Development follows a monodelphic pattern similar to that described by previous workers (Hirschmann, 1962; Hechler and Taylor, 1966a). No evidence of a vestigial ovary as observed by Roman and Hirschmann (1969) in Pratylenchus Filipjev, 1936, was seen. A single germinal nucleus in the genital primordium of second stage juveniles of some monodelphic species has been noted (Hirschmann, 1962; Anderson and Darling, 1964a), whereas several didelphic species have two germinal nuclei in the genital primordium (see Van Weerdt, 1960; Chuang, 1962; Dasgupta and Raski, 1968; Hirschmann and Triantaphyllou, 1968). However, in addition to T. rikus other monodelphic species are known to possess two germinal nuclei (see Hechler, 1963; Hechler and Taylor, 1966a). I am therefore in agreement with Hirschmann (1962) in considering that there is no correlation between the number of gonads in the adult and the number germinal nuclei.

The moulting process of T. rikus occurs in four stages: initial inactivity; formation of the new stylet and cuticle; separation of the old cuticle; and finally rupture of the cast cuticle. The pattern is similar to that of the other nematodes. Moulting of the old stylet and sclerotisation of the new one follows the same sequence as described for Radopholus similis (Cobb, 1893) Thorne, 1949 (Van Weerdt, 1960) and Pratylenchus spp., (Roman and Hirschmann, 1969).

No specific cells were involved in stylet formation in T. rikus. Anderson and Darling (1964b) observed sclerotised rings in the stylet region of Ditylenchus destructor Thorne, 1945, which they considered to function in the formation of the stylet. Roman and Hirschmann (1969) noted similar structures in Pratylenchus but concluded that they were connected to the guiding sheath of the stylet and did not recognise any stylet forming function. Pulsation of the median oesophageal bulb was observed in T. rikus prior to exsheathment and the nematode increased in size over the period between separation of the cuticle and escape. Hechler and Taylor (1966b) observed similar pulsations in Seinura Fuchs, 1931, species and suggested that these may be involved in the uptake of fluid, thereby creating an internal pressure and aiding elongation of the nematode. The uptake of fluid could explain the increase in size of T. rikus over the final moulting stage. In Seinura a copious flow of material from the oesophageal gland was seen which preceded softening of the cuticle and exsheathment (Hechler and Taylor, 1966b). This was not observed in T. rikus where the cuticle appeared to become more pliable following active movement of the nematode and probing of the stylet against the inner wall in a manner comparable with that seen just before the eggs hatch. On completion of the moult, the cuticle splits just behind the head and is shed in one piece as in Seinura (Hechler and Taylor, 1966b).

The life-history of T. rikus deviates slightly in chronology from the cycle discussed for A. neozelandicus but is otherwise similar. Moreover, the increase in time required for egg hatch with decreasing temperature is of the same order.

4.5 APHELENCHOIDES BICAUDATUS (IMAMURA, 1931) FIL. and SCH. STEK., 1941

INTRODUCTION

Aphelenchoides bicaudatus (Imamura, 1931) Fil. and Sch. Stek., 1941, was frequently isolated from Broken River soils. The species reproduced on fungi, moss protonemata, root hairs and lucerne callus tissue (see Chapter 3). The mycophagus habit of A. bicaudatus has been previously reported (Siddiqui, 1966; Schoeneweiss, Taylor and Edwards, 1967; Siddiqui and Taylor, 1969). In addition, Siddiqui (1966) described feeding on a yeast and an alga. Because of the short generation time of the nematode, it was well suited for comparisons of host suitability for population development, and for investigations on orientation responses to different host species.

Siddiqui (1966) conducted detailed studies on the biology of A. bicaudatus from turf grass from Illinois, U.S.A. Preliminary observations on the biology of the Broken River isolate indicated that there were some differences between the populations. Studies on aspects of the biology of the Broken River population were carried out to enable further comparison.

METHOD

Host suitability: A wide range of fungus species, plant tissue, and three species of algae were tested in host range studies. For each fungus species four 9.0cm diameter Petri plates containing 20ml of cornmeal agar, were inoculated by streaking. The plates were incubated for five days at 20°C to enable colonies to establish. Ten surface sterilised pre-adult A. bicaudatus were placed

on each plate and the cultures were incubated for a further four weeks before population levels were determined. For each of the species of algae a 2ml suspension of concentrated algae cells was pipetted into four 9.0cm diameter Petri plates. The suspension were dispersed in 18ml of cooled soil extract agar and on solidification of the medium were inoculated with 10 pre-adult A. bicaudatus. Lucerne callus tissue was cultured by the method described in Chapter 3. Sections of callus tissue about 2.0-2.5cm in diameter were asceptically transferred to fresh culture medium in each of four 9.0cm Petri plates, and inoculated with 10 pre-adult A. bicaudatus.

All treatments were incubated at 20°C before population levels were determined. For the fungi and algae cultures nematode numbers were estimated from sub-samples taken from each plate. Eight 9.0mm diameter plugs were drawn from standardised positions on the colonies and macerated in 15ml of water. The suspensions were poured over a nylon gauze sieve (mesh size 68μ). Water was added until the bottom of the gauze was covered with a thin film. After 48 hours the nematode suspension was collected and the total volume made up to 100ml. The nematodes in a 10ml aliquot of the sample were counted. Lucerne callus tissue was removed after four weeks, macerated in water, and the nematodes extracted and counted as described above. The nematode numbers presented in Table 13 are estimates of the total population calculated from the aliquot counts.

Effect of temperature on population development: The effect of incubation at 5, 10, 15, 20, and 25°C (the range of temperatures used for egg development studies) on population development of A. bicaudatus was investigated. U. atrum was used as the host fungus. Petri plates containing 20ml of cornmeal agar were inoculated by streaking

the surface of the agar with a suspension of conidia. The cultures were incubated for 48 hours at 25°C to promote mycelial growth before each was inoculated with 10 pre-adult A. bicaudatus. Following inoculation, four plates were incubated for four weeks at each temperature. Population levels at the end of this period were estimated using the method described above.

To ensure that food was not the primary limiting factor, the effect of temperature on the growth of the fungus alone was measured at the same time. Plugs of agar 3mm in diameter were removed from the periphery of colonies of U. atrum and placed in an inverted position in the centre of Petri plates containing cornmeal agar. Brancato and Golding (1953) established that colony diameter is a valid measure of the effects of environmental factors on fungus growth. Hence after eight days incubation at each temperature colony growth was measured for four isolates per treatment by taking the average of two diametrical measurements made at right angles to each other.

Host attraction: Experiments were carried out to investigate the effects of host location on movement of A. bicaudatus under culture conditions, and to investigate possible differences in 'attractiveness' between hosts to the nematodes which may be of significance in the interpretation of distribution patterns expressed in the field.

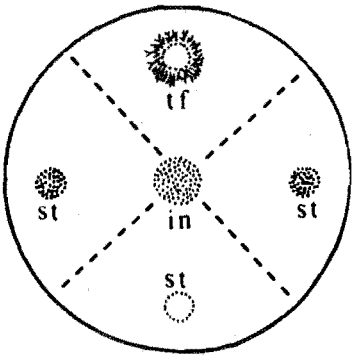
i) Orientation response to fungi: Nine centimetre diameter Petri plates were poured with approximately 15ml of cornmeal agar. Seven millimetre diameter discs were cut from two week old cultures of U. atrum, Rhizoctonia solani, Fusarium oxysporum, Sclerotinia sclerotiorum, Pythium sp., Trichothecium roseum and Zygorhynchus moelleri were placed on the surface of the cornmeal agar,

2.0cm from the rim of the plate (Figure 21A). A sterile cornmeal agar disc was placed 2.0cm from the end of a transect from the disc of fungus inoculum, drawn through the centre of the dish. Two sterile filter paper discs were placed 2.0cm from each end of a second transect drawn at an angle of 90° to the first (Figure 21A). The plates were incubated for 24 hours at 24°C to promote growth of the fungus before they were inoculated with A. bicaudatus. Inoculation was achieved by pipetting a concentrated suspension of sterile nematodes onto 1.0cm diameter sterile filter paper discs, which were then placed in the centre of each Petri plate. The plates were divided into quadrats and incubated for 18 hours at 20°C before the number of nematodes in each sector was counted. Four replicates for each fungus species were examined, and the significance of the deviation from a random distribution tested using Chi-square analysis.

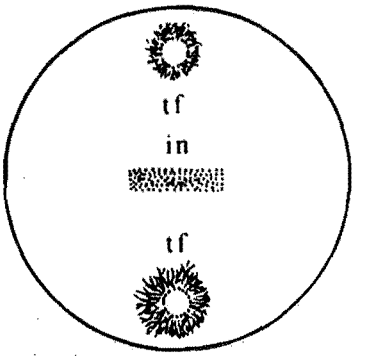
ii) Differential attractiveness of fungi: Experiments were carried out to establish if A. bicaudatus is attracted differentially to specific fungi; to determine whether or not host selection occurs in nematodes under the influence of a multiple stimulus from several species of fungi, and to investigate the movement of A. bicaudatus feeding on one fungus species growing in close proximity to a second species.

In the first experiment the 'attractiveness' of Pythium sp., T. roseum, Z. moelleri, S. sclerotiorum, F. oxysporum and R. solani was

FIGURE 21. Experimental design of host attraction experiments using Aphelenchoides bicaudatus. A) Orientation response to fungi. B) Differential attractiveness of two fungi. C) Multiple choice experiment. D) Influence of host fungi growing in close proximity.



A



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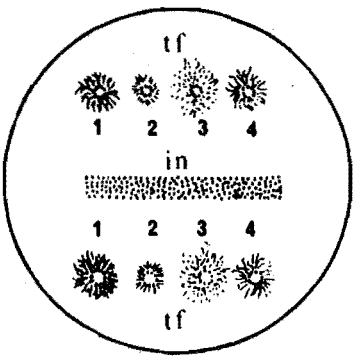

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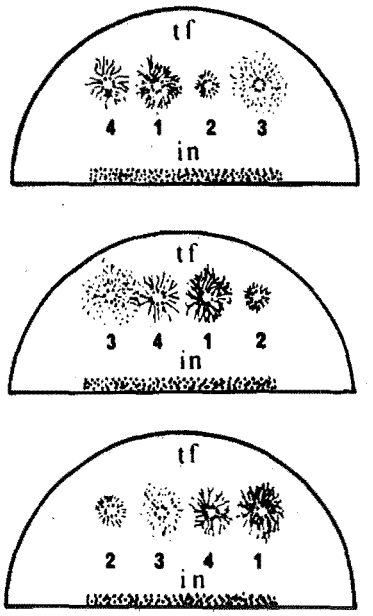
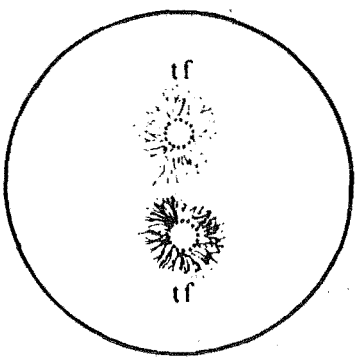




D

Filter paper
st sterile
in inoculated

Media disc
st sterile
tf test fungus

tested against U. atrum. A 7.0mm disc of the test fungus was placed 2.0cm from the perimeter of a Petri plate containing 15ml of cornmeal agar. On the opposite side of the plate, 2.0cm from the perimeter, a 7.0mm disc of U. atrum was placed. Each treatment was replicated four times. The plates were incubated for 48 hours at 24°C before sterile filter paper strips (20.0 x 4.0mm), inoculated with sterile A. bicaudatus were placed at the midpoint between the fungus colonies (Figure 21B). After incubation for 18 hours at 20°C, the number of nematodes feeding within each colony was determined.

In the second experiment 3.0mm diameter discs of F. oxysporum, U. atrum, T. roseum and S. sclerotiorum were placed 1.0cm apart in the combinations shown in Figure 21C, at each end of Petri plates containing cornmeal agar. The plates were incubated for 48 hours at 24°C before nematode infested filter paper strips (45.0 x 4.0mm) were placed between the rows of test fungi: After incubation for 18 hours at 20°C, the number of nematodes feeding within the perimeter of each colony was counted.

In the third experiment 7.0mm diameter discs of F. oxysporum, U. atrum, S. sclerotiorum and T. roseum, were placed 10.0mm apart from discs of U. atrum. Two plates for each combination were incubated for 24 hours at 24°C and then alternate colonies in each species combination were inoculated with 15 A. bicaudatus (Figure 21D). The number of nematodes feeding on each colony was counted after incubation of

the plates for 48 hours at 20°C.

iii) Orientation response to seedlings: Surface sterilised short rotation ryegrass seeds were germinated in 1.0% water agar. When the emerging radicles were about 2.0cm long, 20 surface sterilised A. bicaudatus were introduced about 1.0cm from them by placing individual animals into the side of small wells cut into the agar. The experiment was repeated four times and the number on nematodes feeding in the root hair zone or on the main root was recorded 12 hours after inoculation.

iv) Differential attractiveness of fungi and seedlings: Surface sterilised short rotation ryegrass seeds were germinated in 1.0% water agar. When the radicles were 1.0-2.0cm long, a 0.7mm diameter core of agar was removed from a position about 3.0cm from the seedling and replaced with a core of the same size from a water agar culture of U. atrum. The plates were incubated on an angle to promote root growth along an axis at 90° to the developing fungus colony. After 48 hours incubation, 20 surface sterilised A. bicaudatus were placed in wells at the midpoint between the seedling and the fungus. The position of the nematodes was recorded after 12 hours.

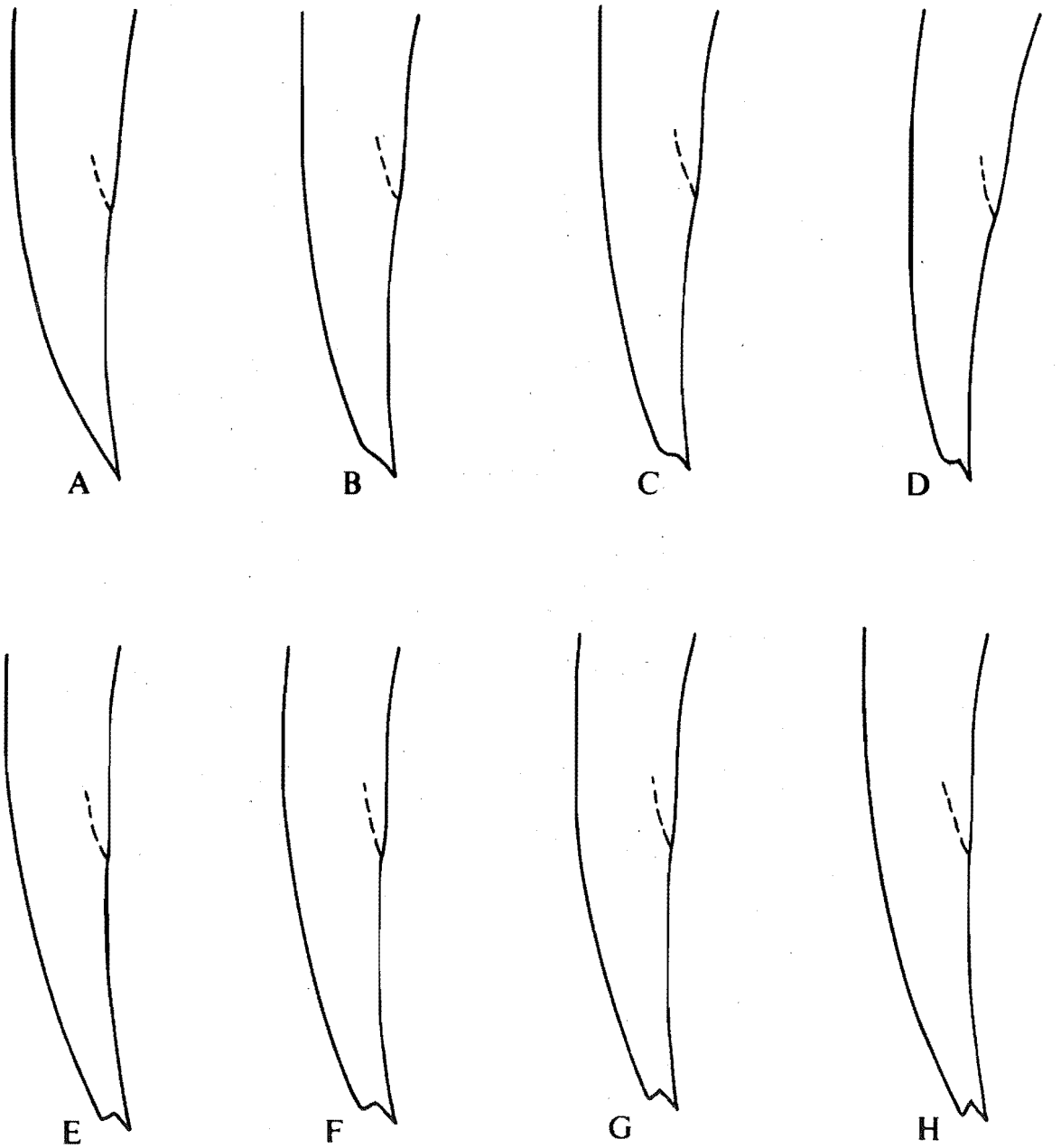
RESULTS

MORPHOLOGY: Measurements: Specimens mounted in glycerine. Females (30): L = 400-569μ (461); a = 25.3-29.7 (27.2); b = 6.9-8.9 (7.6); c = 12.7-15.8 (14.2); V = 65.8-77.0%

(70.8); stylet = 10.0-11.0μ (10.4).

On the basis of Sanwal's (1961) key to the species of the genus Aphelenchoides Fischer, 1894, the Broken River isolate was initially considered to be closely related to Aphelenchoides subtenuis (Cobb, 1926) Steiner and Buhrer, 1932. However, apart from tail shape and the presence of a phasmid, the morphological characters agree with the detailed description of A. bicaudatus given by Siddiqui, (1966). Although Siddiqui (1966) did not comment on variability of tail shape in adult animals, he reported a range of forms in juveniles of the species, and ventrally mucronate tail without bifurcation for adult males. Sanwal (1965a) noted that considerable variation in the shape of tail termini may occur within populations of Aphelenchoides species, but recognised two distinct types of tail form in species closely related to Aphelenchoides parietinus (Bastian, 1865) Steiner, 1932 (Sanwal, 1965b). About one-half of the specimens of the present isolate, including most juveniles, characterise Sanwal's (1965b) first general shape in possessing a bluntly rounded terminus with a small ventral mucro; the remainder exhibiting varying degree of bifurcation (Figure 22A-H). Siddiqui (1966) did not observe a mucro on the tails of juveniles of his specimens whereas most juveniles of the present isolate possessed a fine ventrally located mucro which was visible at high magnifications. But because of the absence of a mucro on the tails of some juveniles, and the general variability of tail shape in adults from populations raised from single females, tail shape is not considered to be a sufficiently stable character to justify species differentiation of the Broken River isolate.

FIGURE 22. Variation in tail shape of *Aphelenchoides bicaudatus*.


A
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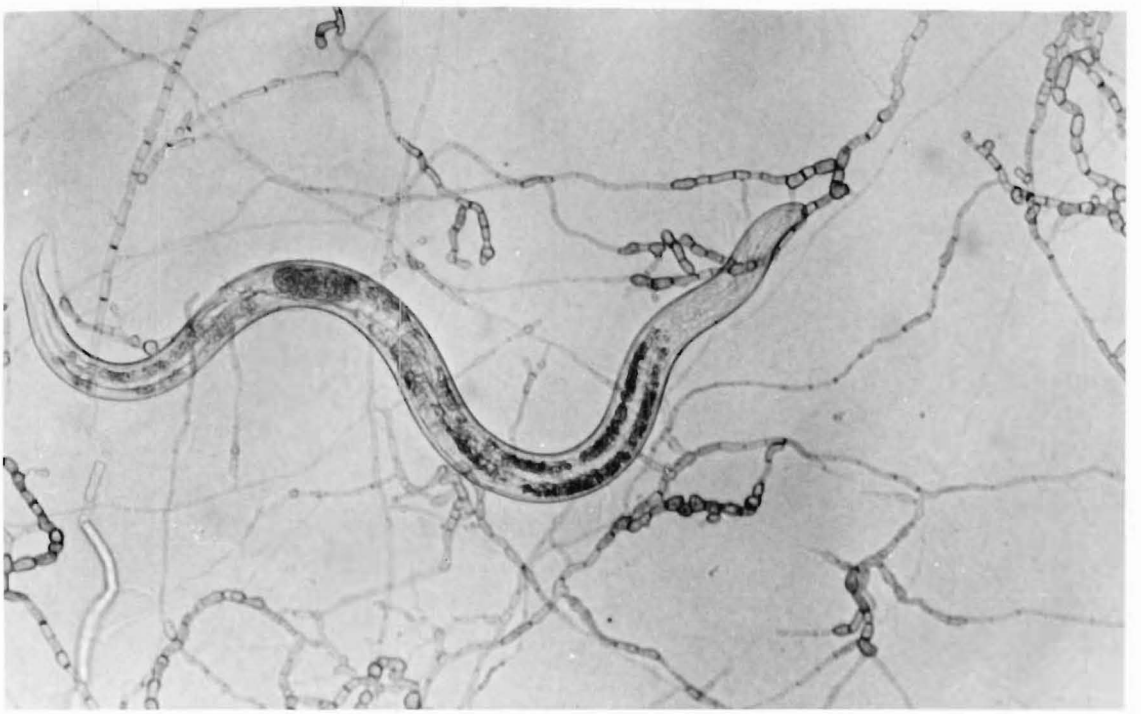
PENETRATION AND FEEDING: A. bicaudatus moves sinuously through the medium, 'nosing' along a host filament, making occasional tentative jabs with its stylet (Figure 23). As penetration occurs the lips are pushed against the host cell causing an indentation of the wall and rapid movements of the stylet follow. The pre-penetration activity lasts about one second on fungus hyphae, but may take up to 12 seconds on plant root hairs. During penetration the stylet is positioned at approximately 90^{o} to the longitudinal axis of the host cell, even when the head of the nematode is at a more acute angle. The stylet is inserted a distance of about one-fifth of its length.

Feeding activity is initiated by a vibratory movement of the anterior portion of the median oesophageal valve. On the fungus U. atrum the feeding periods ranged from 10-65 seconds, with 15 seconds to nine minutes between periods. During ingestion, movement of the metacorpal valve plates is rapid (about two pulsations per second). Movement of hyphal protoplasm toward the spear orifice occurs, and on extraction of the stylet the cells are quite flaccid. Pulsation of the metacorpal valve plates continues until the stylet is extracted. Nematodes sometimes have difficulty in removing their stylets from the collapsed host cell, in which cases violent sideways movements of the head are necessary to facilitate extraction. On plant root hairs feeding periods were usually longer, lasting up to seven minutes. Pulsation of the metacorpal valve plates was slower; about one pulsation per second. Although there was no evidence of secretory activity during feeding, host protoplasm was seen to move toward the stylet orifice which resulted in a slight build-up of material about the tip.

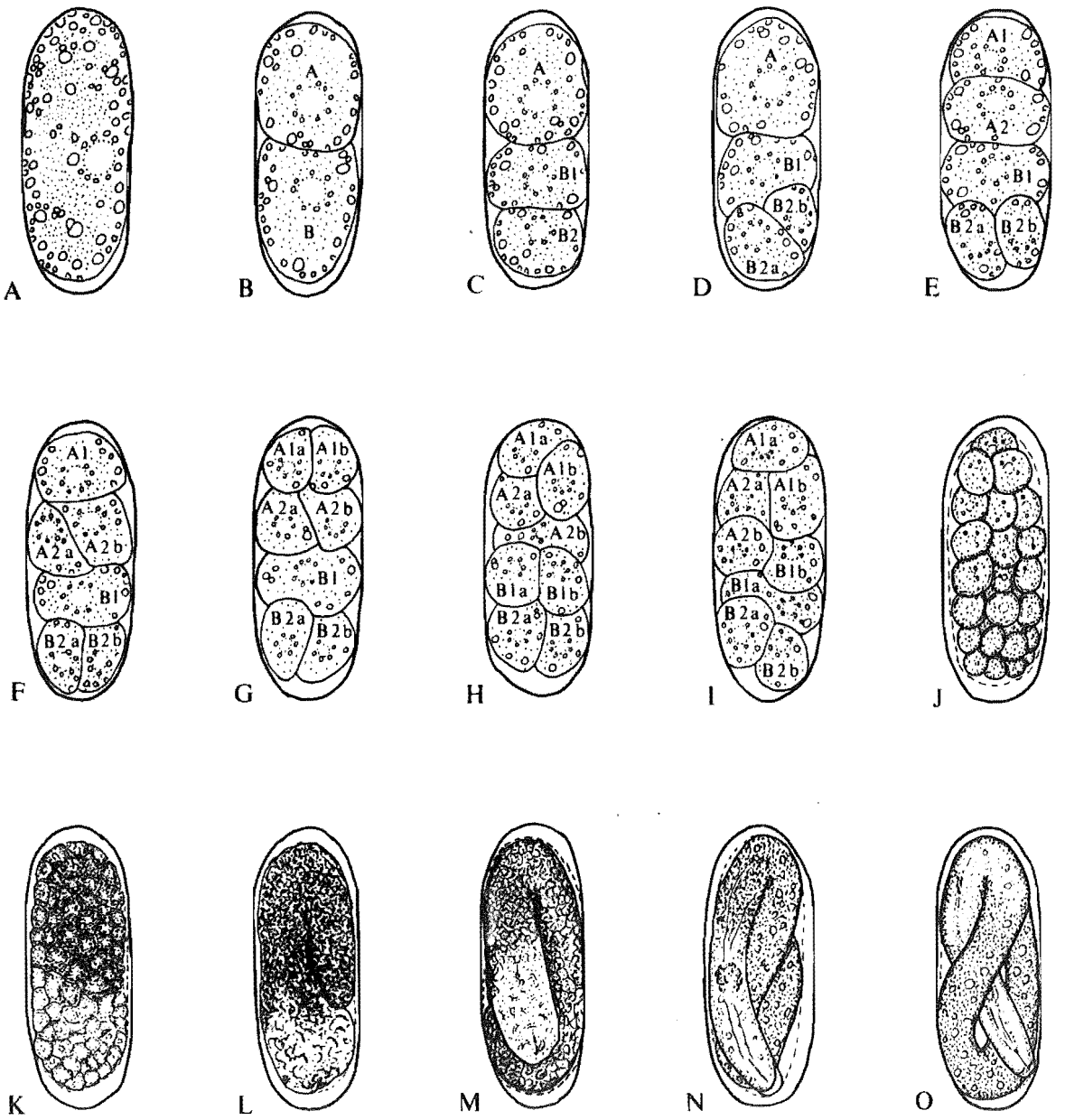
FIGURE 23. Gravid *Aphelenchoides bicaudatus* feeding on a hypha of *Ulocladium atrum*.



HOST RANGE: All of the fungi tested served as hosts for A. bicaudatus, but the population levels reached over the four week incubation period varied considerable between hosts (Table 13). Populations developed on lucerne callus tissue, but nematodes did not survive on Chlorella vulgaris, Chlamydomonas sp., or Protosiphon sp.

LIFE-HISTORY: Embryonic development: Eggs measure 36-46μ x 18-22μ at laying; they are oval, bluntly rounded, with a smooth shell (Figure 24A). On some occasions giant eggs measuring 58-64μ x 20-22μ are laid, but development within these eggs was not observed to proceed past the tadpole stage. In the normal egg the first cleavage, at right angles to the longitudinal axis of the egg at about 55% of its length, results in two slightly unequal blastomeres (Figure 24B) about one to five hours after laying. The larger posterior cell divides one to two hours later (Figure 24C). Division of cell B2 follows (Figure 24D) and after migration of daughter cell B2b to a more anteriad position (Figure 24E), division of A2 and A1 occurs (Figure 24F and 24G). The eight cell stage (Figure 24H) is reached after nine to 11 hours. Migration of cells during the eight cell stage occurs (Figure 24I) and continues throughout the following stages. Subsequent divisions are difficult to follow accurately (e.g. Figures 24J and 24K). Approximately 30-35 hours from laying, a fold appears in the embryo about two-thirds of the egg length from the posterior end (Figure 24L) marking the development of the tadpole stage. Movement begins about 40-45 hours from laying when the juvenile is about twice the egg length long. At this stage the head is broad and more hyaline in appearance (Figure 24M). The juvenile is well developed approximately 60 hours after laying,

FIGURE 24. Embryology of Aphelenchoides bicaudatus (see text for explanation).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A
B
B
A
B1
B2
C
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B1
B2b
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D
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A2
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B2b
E
A1
A2a
A2b
B1
B2a
B2b
F
A1a
A1b
A2a
A2b
B1
B2a
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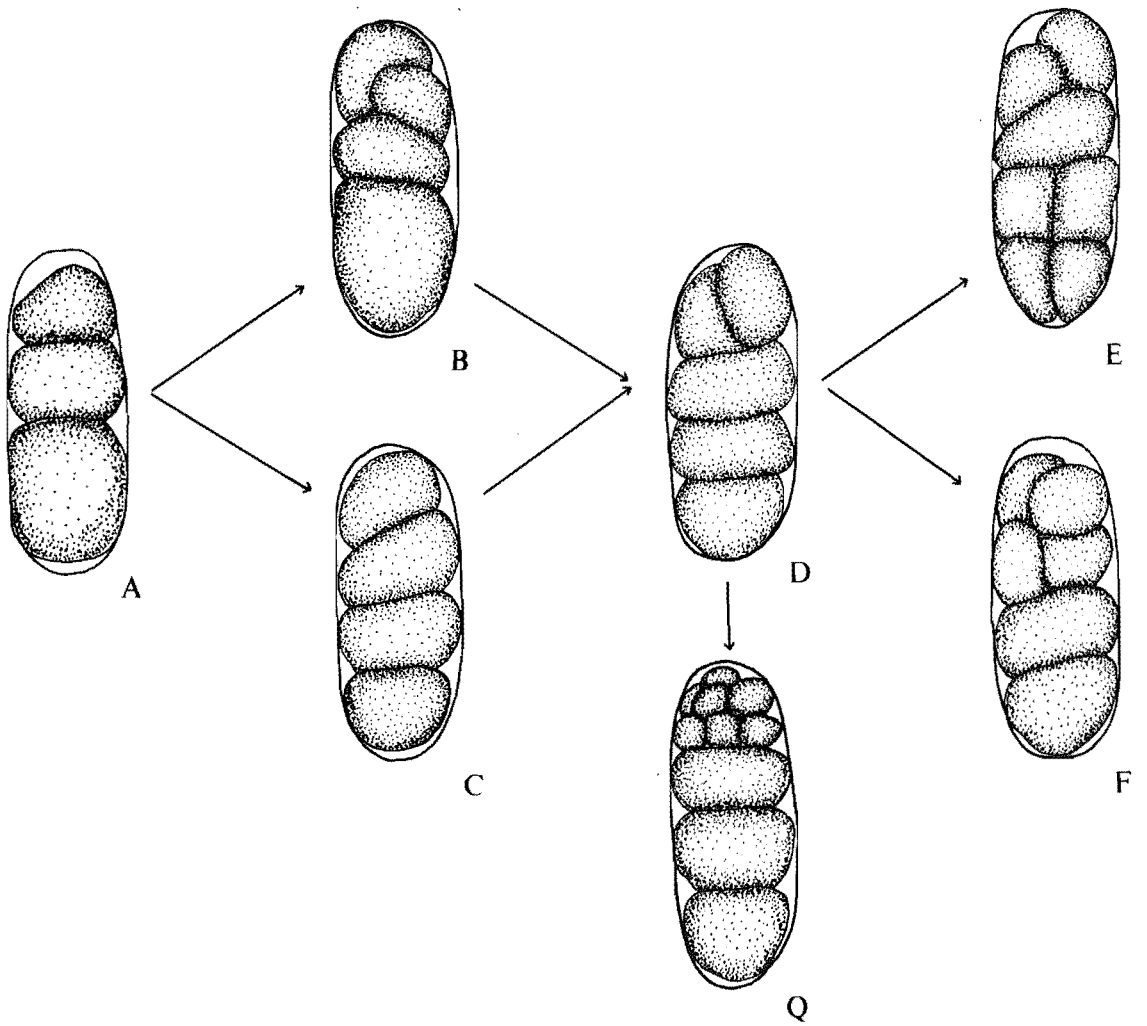
but the cephalic framework is weakly formed and the stylet appears as a faint refractive line. Movement slows down over the next few hours and finally ceases. Separation of the cuticle in the head region just posterior to the lips occurs as the juvenile undergoes the first moult within the egg (Figure 24N). The second stage juvenile (Figure 24O), hatches 70-95 hours after laying (15 observations).

Variation in the pattern of early blastomere divisions: In 14 of 15 eggs studied in sequence, division of blastomeres to the eight cell stage followed the pattern illustrated in Figure 24. However, on some occasions division of eggs in stock cultures was seen to vary from this pattern (e.g. Figure 25A-Q). Subsequent development of all but the stage shown in Figure 25Q, appeared normal and juvenile A. bicaudatus hatched.

Hatching: Until the first moult, the vitelline membrane was readily observed containing the mobile first stage juvenile within the egg. Following the moult, the membrane was difficult to discern and ultimately could not be seen. The time of its disappearance seemed to coincide with the induction of shell distortion. Continued pressure from the active juvenile applied by curvature of the cephalic region and tail region and accompanied by stylet probing against the cell wall was associated with an increase in egg dimensions of about 9.0-17.0% during the last hour before hatching occurred. The juvenile emerges by stretching the wall until it ruptures, or by extending the wall and bursting it with a single jab of the stylet tip.

Post-embryonic development: A. bicaudatus undergoes three further moults before maturing. The second moult occurs about 30-40 hours after hatching, the third approximately 50-58 hours later, and the fourth moult is completed

FIGURE 25. Variations of early blastomere divisions of Aphelenchoides bicaudatus.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C
D
E
F
Q

TABLE 13: Population development of A.bicaudatus on a range of hosts

Host	Species	Mean number of nematodes
Fungus	Pythium sp.	410
	Chaetomium globosum	890
	Trichoderma viride	1,080
	Mucor sp.	1,180
	Phytopthora cactorum	2,830
	Cladosporium sp.	3,230
	Trichothecium roseum	3,880
	Aspergillus niger	4,140
	Verticillium sp.	13,720
	Mortierella alpina	18,360
	Zygorrhynchus moelleri	31,340
	Penicillium sp.	54,110
	Gliocladium roseum	61,240
	Ascochyta sp.	63,950
	Sclerotinia sclerotiorum	75,340
	Botrytis cinerea	86,060
	Alternaria brassicicola	105,000
	Stemphylium botryosum	121,280
	Fusarium oxysporum	128,130
	Agaricus bisporus	131,920
	Phoma sp.	156,260
	Pleiochaeta setosa	176,000
	Rhizoctonia sp.	179,080
	Ulocladium atrum	199,250
	Rhizoctonia solani	225,740
Algae	Chlorella vulgaris	0
	Chlamydomonas sp.	0
	Protosiphon sp.	0
Plant callus tissue	Medicago sativa	111,240

after a further 57-77 hours. The generation time on U. atrum at 20°C is 220-280 hours, but varies considerably with temperature and the condition of the host. On old cultures in a degenerative condition, or on cultures contaminated by bacteria the generation time may be doubled.

Adult: The first egg is laid about 10 hours after the adult completes the final moult. Males were never observed. The rate of laying increases from about one egg every six hours on the first day to one every two to three hours by the fourth day. Laying continued for 22-33 days for females transferred at weekly intervals to fresh cultures of U. atrum but the laying period is terminated prematurely in old cultures. Under the latter conditions, some females survived for three weeks without laying eggs.

EFFECT OF TEMPERATURE ON EGG HATCHING AND POPULATION DEVELOPMENT: Egg hatching: None of the eggs incubated at 5°C hatched within 30 days. Six of 11 eggs hatched after $14\frac{1}{2}$ - 17 days at 10°C; development of the remainder appeared to be arrested at about the tadpole stage. All eggs hatched within seven and one-half to nine days at 15°C, three to three and one-half days at 20°C, and two and one-half to three and one-half days at 25°C

Population development: The rate of population development increased with increasing temperature (Table 14).

TABLE 14: Effect of temperature on population development of A. bicaudatus and colony growth of U. atrum.

Temperature °C	Mean number of A. bicaudatus after four weeks	Mean colony diameter (cm) of U. atrum after 8 days
5	0	2.75
10	40	3.12
15	4810	4.40
20	98250	6.05
25	85165	7.70

HOST ATTRACTION: Orientation to fungi: A. bicaudatus was attracted to all of the fungus species tested (Table 15). Most nematodes reached the fungus colony within eight hours of inoculation. Initial patterns of movement appeared to be random, but when an individual approached to within about 10-15mm of the mycelium orientation toward the fungus colony occurred.

Differential attractiveness of fungi: The results summarised in Table 16 show that fungus species growing under culture conditions may exhibit different powers of attraction to A. bicaudatus. In all comparisons nematodes were observed feeding on both of the fungus colonies. Hence some animals, particularly those along the margins of the filter paper strips may not have been subject to the influence of both of the test colonies.

In the second experiment, the reaction of A. bicaudatus

TABLE 15: Effect of the location of fungus colonies on the distribution of A. bicaudatus under culture conditions.

Fungus species	X^2
Pythium sp.	103.9
Trichothecium roseum	294.9
Zygorhynchus moelleri	174.1
Sclerotinia sclerotiorum	59.9
Fusarium oxysporum	340.9
Ulocladium atrum	321.6
Rhizoctonia solani	283.5

X^2

$(P_{0.01}) = 6.63$

TABLE 16: Differential attraction of A. bicaudatus to fungus species growing under culture conditions.

Attractiveness of U. atrum compared with		test fungi	X^2
U. atrum	=	Pythium sp.	0.22
U. atrum	>	T. roseum	4.65*
U. atrum	=	Z. moelleri	0.15
U. atrum	>	S. sclerotiorum	23.81**
U. atrum	<	F. oxysporum	7.77**
U. atrum	>	R. solani	10.69**

X^2

$(P_{0.01}) = 6.63$**

$(P_{0.05}) = 3.84$*

to a multiple stimulus from each direction supplied by colonies of several fungi was tested. The results followed a similar pattern to those of the previous experiment. There was a significant departure from a random distribution (X^2 = 728.25; $X^2(P<0.01)$ = 6.63, with a preference shown for F. oxysporum > U. atrum > S. sclerotiorum > T. roseum. This indicated that the different fungi may produce specific attractants. But the mean colony diameter of each species decreased in a corresponding order to 'attractiveness' (e.g. mean colony diameter of F. oxysporum = 15mm; U. atrum = 11mm; S. sclerotiorum = 9 mm; T. roseum = 6mm), which suggested that the intensity of the attraction stimulus may depend on the growth rate of the fungus, or that a large colony diameter proportionately increases the chances of contact during an orientation response of nematodes to a common stimulus.

If a specific attractant was produced by each fungus, it could be expected that migration of nematodes from one colony to a more 'attractive' colony would occur when different species of fungi were growing in close proximity to one another. This was not shown in the third experiment (Table 17), despite the fact that at the time of counting peripheral growth of some colonies overlapped.

Attraction to seedlings: Eighty percent of the nematodes placed 1.0cm from the roots of ryegrass seedlings were observed feeding within the root zone after 12 hours. Movement was directed toward the roots and about 50% of the individuals reached the radicles after one hour.

Differential attractiveness of fungi and seedlings: There was no apparent preference for either ryegrass seedling roots or U. atrum mycelium under culture conditions. A. bicaudatus was observed feeding on both hosts but the distribution of nematodes between the hosts was not significantly different (X^2 = 1.88; X^2 ($P<0.01$) = 6.63).

TABLE 17: Location of A. bicaudatus after incubation in the presence of paired fungus hosts.

Fungus species combination	Nematode numbers	
	Plate one	Plate two
F. oxysporum	3	13*
U. atrum	12*	1
S. sclerotiorum	2	11*
U. atrum	12*	3
T. roseum	4	10*
U. atrum	11*	5
U. atrum	2	11*
U. atrum	10*	4

* = the inoculated colony of each pair

DISCUSSION

No host-penetration prior to the onset of feeding was observed for A. bicaudatus. Immediate ingestion of cell contents following penetration has been noted several Aphelenchoides species (Christie and Arndt, 1936; Christie and Crossman, 1936; Steiner, 1936; Siddiqui, 1966).

The wide host range reported by Siddiqui (1966) was confirmed and extended in the present study. However, in contrast to Siddiqui's isolate, A. bicaudatus from Broken River did not reproduce on cultures of algae, but plant root hairs and plant callus tissue were suitable hosts. Several Aphelenchoides species are acknowledged facultative higher plant and fungus feeders (e.g. A. fragariae

(Christie and Crossman, 1936), A. bessyi (Todd and Atkins, 1958), and A. blastophthorus (see Franklin, 1952, and Hooper, 1963). The nematode reproduced on all of the fungi tested. Consistent with Siddiqui's (1966) trials, Phycomycete fungi are generally poorer hosts than many species of Ascomyctes, Basidiomycetes and Deuteromycetes.

Unequal division of blastomeres of A. bicaudatus at the first cleavage is similar to other nematodes (Van Weerdt, 1960; Yuksel, 1960; Chuang, 1962; Thomas, 1965; Hechler and Taylor, 1966a) and was reported by Siddiqui (1966). The pattern of subsequent divisions differed slightly from that described by Siddiqui (1966). Siddiqui (1966) reported that mitosis was delayed in the larger blastomere until the smaller cell had given rise to four daughter cells, or the five cells stage was reached. In the Broken River isolate division of the large cell (B., Figure 24B and C), occurred first, followed by division of B2 and then A, to reach the five cell stage, but variations were noted. Siddiqui (1966) also reported one instance of variation from the usual pattern. In the present study the time required for development of the embryo, and the rate of population development of A. bicaudatus decreased markedly with increased temperature from 10°C to 20°C, but there was little difference between development at 20°C and 25°C. Siddiqui (1966) observed marked reduction of embryonic development time and increased population development up to 28°C.

Dao (1970) demonstrated that populations of nematodes may be temperature-adapted to their climate, thus emphasising the value of the biological species concept in work with nematodes. Adaption of such a nature could explain some of the biological differences between A. bicaudatus from Broken River and A. bicaudatus from

Illinois (Siddiqui, 1966). Further, the variation in embryonic development patterns of the Broken River isolate may reflect genetic variability and the possible existence of biological species within a population.

A. bicaudatus was attracted to fungus colonies and to seedling roots growing in culture. Katznelson and Henderson (1962) reported that metabolic by-products of fungi can influence the position which feeding nematodes assume on a fungus colony. Later, attraction of fungus feeding nematodes to colonies of fungi was demonstrated by Townshend (1964). He observed direct rather than random movement of Aphelenchus avenae and Bursaphelenchus fungivorus toward fungus colonies as far as 2cm away, and suggested that quantitative and qualitative variability in chemical composition between fungi may affect the suitability of different hosts. Pillai and Taylor (1967) also noted the differential attraction of mycophagous nematodes to fungi, but the most attractive fungus for a species was not necessarily the most suitable host for population increase. More recently, Klink, Dropkin and Mitchell (1970) showed that Neotylenchus lindfordi Hechler congregated around colonies of fungi. Attraction was strongest towards Gliocladium roseum Bainier. In experiments with G. roseum, the attractants were shown to be small thermostable molecules, soluble in methyl alcohol and unaffected by pH (Klink, Dropkin and Mitchell, 1970).

In multiple choice experiments, evidence suggesting differential host selection was recorded. This was not supported by the results of trials in which nematodes were placed on one colony of a pair growing in close proximity to one another. Some migration between hosts occurred, indicating that A. bicaudatus was not physically held, but the majority of animals remained within the periphery of

the colony on which they were placed. Klink, Dropkin and Mitchell (1970) also doubted the existence of host specific stimulants as they found that N. linfordi responded not only to the filtrate of G. roseum, but to compounds such as NaH_2PO_4, $(NH_4)_2CO_3$ and HCl. Furthermore, nematodes are known to respond to stimuli from both suitable and non-suitable hosts (Dijkstra, 1957; Dropkin and Webb, 1967; Griffen, 1969). If host specific attractants were produced in the present experiments, then migration towards the most attractive colony in each pair of colonies should have occurred. It seems that fungus colonies and seedling roots growing under culture conditions produce attractants of a similar nature. The differential 'attraction power' of fungus species to A. bicaudatus is probably related to the rate of production of the attractive principle.

4.6 SEINURA DEMANI (GOODEY, 1928) J. B. GOODEY, 1960.

INTRODUCTION

Seinura demani (Goodey, 1928) J.B.Goodey, 1960, was isolated by seeding Petri plates containing cornmeal agar with top-soil from Broken River. The predaceous habit of Seinura species was described by Lindford (1937a). and Lindford and Oliviera (1937). Later, Hechler (1963), Hechler and Taylor (1965, 1966a, 1966b), carried out detailed biological studies on a number of species of the genus, but S. demani was not included. Because of the short generation time characteristics of Seinura species, the biology of S. demani was considered as an example of a predacious species.

During preliminary observations male S. demani were frequently observed in close association with pre-adult moulting females for several hours before the exsheathment which indicated that an attraction mechanism may have been operative. The involvement of attraction in the location of mates in nematodes has come to the attention of nematologists over the last decade (e.g. Greet, 1964; Jones, 1966; Green, 1966; Bonner and Etges, 1967; Greet, Green and Poulton, 1968; Chin and Taylor, 1969). Experiments were carried out to determine whether attractants are involved in mate location in S. demani.

METHOD

Host range and feeding: Observations on host range and feeding were made on animals cultured in glass ring chambers. Mycophagous test prey were cultured on U. atrum and R. solani, bacteriophagous species were 'started' with a small amount of substrate from the stock cultures, or cultured on E. coli. In addition nematodes were observed in the original soil cultures. Blocks of agar approximately $2cm^2$ were removed and replaced with cooled 1.0% water agar. Prey and predators accumulated in the fresh medium and were studied by inverting the Petri plate on a microscope stage and focussing through the bottom.

Mating behaviour and sex attraction: Observations on the mating behaviour of S. demani were on animals in stock cultures and in culture chambers.

Attraction of males to females was tested in a series of experiments carried out in Petri plates or culture chambers containing a thin layer of 1.0% water agar. Movement was followed for 20 minutes after the introduction of males, and the location of males noted an hour later using a stereo binocular under natural light.

i) Attraction between active adults: Greet (1964) observed a mutual attraction between males and females of Panagrolaimus rigidus (Schneider, 1866) Thorne, 1937. To investigate possible attraction between adult S. demani, well fed mature males were placed on the tracks of females both on the surface and embedded in the agar. In addition, 15 mature females were introduced into a layer of agar on a 15mm^2 cellophane film positioned on the surface of the water agar. After four hours 10 males were placed in the medium about 1.0mm from the edge of the film, and their movement followed.

ii) Attraction of pre-adult moulting females to adult males: Pre-adult moulting females were located in culture chambers and the activity of males entering the vicinity plotted. Green (1966) demonstrated a chemical attraction of male Heterodera rostochiensis Wollenwaber, 1923, and H. schactii Schmidt, 1871, to their females from at least 5.0mm. An experiment was carried out to investigate the effective range of attraction of pre-adult S. demani under culture conditions. Moulting pre-adult females were rinsed in sterile water and transferred to water agar in close groups of five. Three groups were placed on the surface of the medium and three groups were embedded in the medium. After four hours, five males per treatment were placed approximately 2.0mm from two groups, 1.0mm from two, and 0.5mm from two.

iii) Stimulus perception: Green (1967) obtained no orientation response from Heterodera males to

to females when they were separated by a small air space. Hence gustatory, rather than olfactory stimulus perception by males was suggested. A similar experiment was carried out using S. demani. Three groups of five moulting females were placed on thin films of agar on 22mm^2 cover glasses suspended over an agar surface so that less than 1.0mm separated the interfaces. After four hours males were placed beneath the edge of the coverslip.

Sex ratio: Wallace (1963) suggested that the sex ratio of bisexual nematodes is density dependent and possibly controlled by nutrition. The effect of prey abundance on the ratio of male to female S. demani was tested. Three treatments, each with two replicates were set up using culture chambers. In treatments one and two, cells contained water agar, and in three, dense populations of Aphelenchoides bicaudatus feeding on U. atrum. Each replicate was inoculated with 15 female and 10 male S.demani. Approximately 100 prey animals were pipetted into treatments one and two on the first day, with subsequent inoculations every second day for the first five days, and daily there after until the tenth day. Cultures from treatments one and three were extracted in 10ml of water agar after 11 days and the sex determined for 50 adults taken at random. Treatment two was harvested after 20 days without further addition of prey.

RESULTS

HOST RANGE AND FEEDING: S. demani does not differentiate between prey species other than individuals of its own kind. Cannabalism only occurs in starved populations.

Sub-populations were reared on Acrobeloides sp., Chiloplacus sp., two species of Plectus, Wilsonema otophorum (de Man, 1880) Cobb, 1913, Eucephalobus sp., Tylenchus rikus, A. bicaudatus, and Aphelenchus avenae Bastian, 1865. In addition, S. demani was observed feeding on juvenile Tylencholaimus sp. Some prey nematodes are more readily captured than others and in this respect are considered to be better 'hosts'; W. otophorum which is relatively fast moving and characteristically feeds on the surface of the medium, survived more attempted penetrations than did A. avenae. Attemps to kill adult Tylencholaimus sp., were nearly always unsuccessful because the intended victim was able to take evasive action before the stylet penetrated its cuticle.

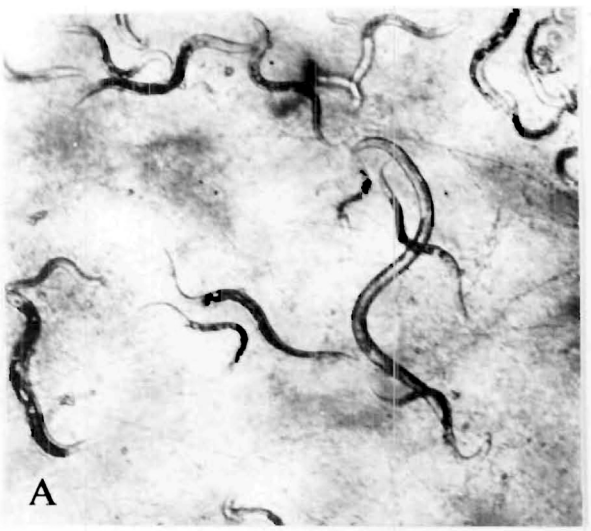
S. demani shows no ability to track prey. The predatory animal recognises another animal only on contact and responds to the stimulus with repeated thrusts of its stylet against the body of the intended victim. The prey nematode is immobilised almost immediately, irrespective of where the penetration is achieved. An oesophageal gland secretion is injected into the prey animal as described by Hechler (1963), and feeding follows (Figure 26A-B).

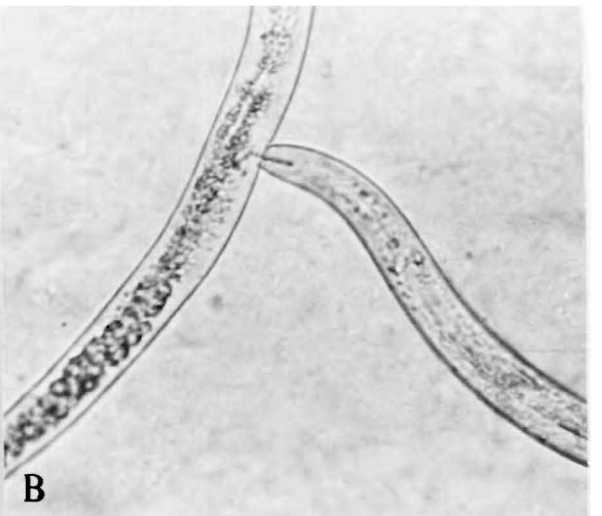
Seinura species require a semi-solid medium such as agar to provide purchase for penetration of prey (Hechler, 1963). S. demani requires similar traction for both penetration and removal of its stylet from victims; when feeding animals are placed in water, they are unable to withdraw their stylets from the cuticle of cadavers.

LIFE-HISTORY:

Embryonic development: Eggs measure 71.0-84.0μ x 19.0-21.0μ at the time of laying. They are straight to gently curved, unsegmented, finely sculptured, and tapered

FIGURE 26. Feeding and mating behaviour of *Seinura demani*.
A) Adult female attacking bacterial feeding nematodes on the surface of a culture. B) Ingestion of victim's body contents. C) Four male *S. demani* in association with a moulting pre-adult female (mf= moulting female). D) Excited male in copulatory position around the body of a female nearing the end of the moult.


A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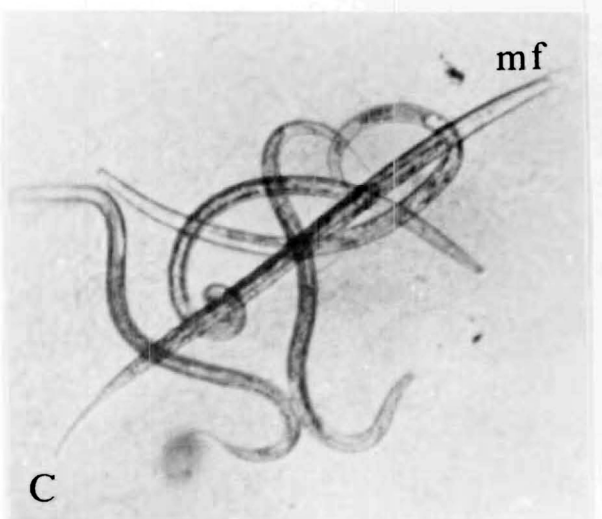
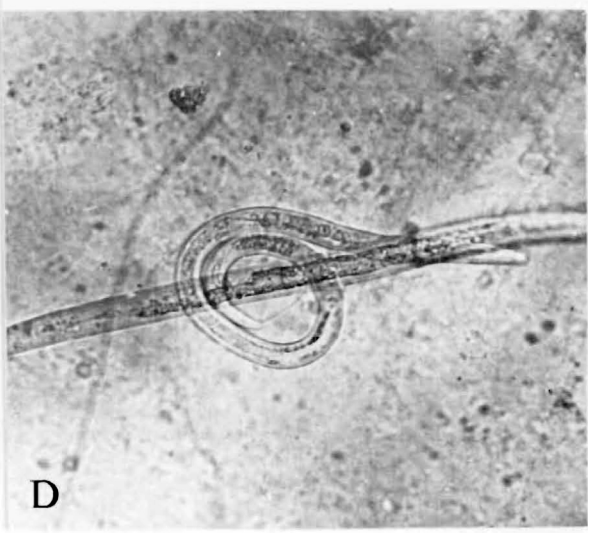
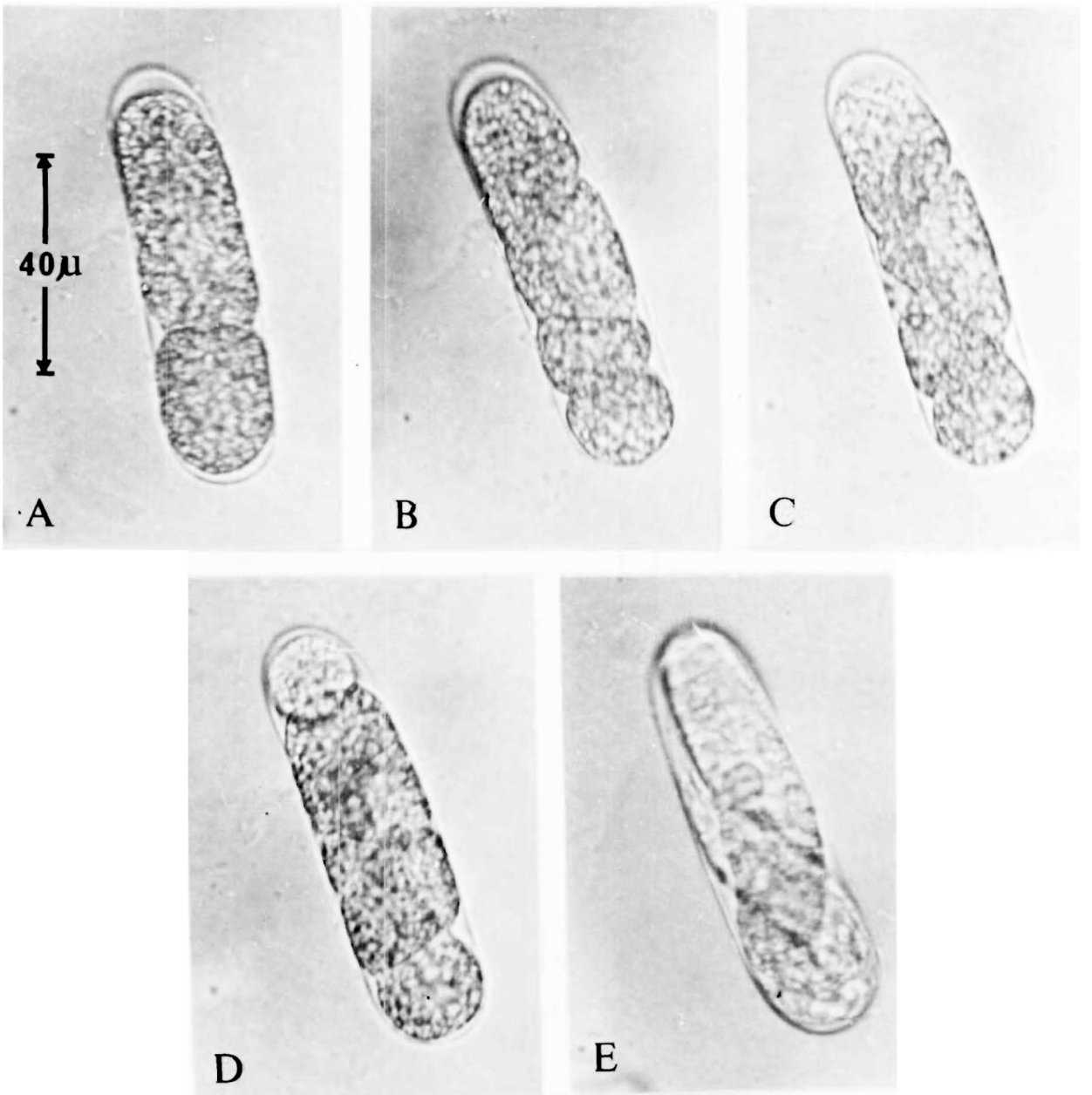

mf
C


D

slightly at the anterior end. Fifteen to 30 minutes after the egg is laid the protoplast contracts to about 92% of its original length. The first division occurs 40-60 minutes after laying and results in two unequal blastomeres (Figure 27A). The larger cell composing about two-thirds of the total protoplast and situated at the anterior end of the egg, divides 30-35 minutes later. Division of the smaller cell follows in two to five minutes, to give a row of cells (Figure 27B). The blastomere positions undergo some reorganisation over the next 10 minutes (Figure 27C). Both of the anterior cells then divide in quick succession (Figure 27D). Further divisions are rapid, making accurate observation difficult.

First stage: About 17 hours after laying, the embryo is approximately twice the length of the egg and movement is almost continuous (Figure 26E). The head of the embryonic nematode develops in the narrow end of the egg. Twenty-two hours after laying, the stylet position is delimited as a faint line as the animal enters the moulting phase. During the inactive period of the moult the oesophageal valve musculature and valve plates become apparent. At this stage the stylet is well defined, but does not appear to be heavily sclerotised. The moulting process involves a series of lateral twitching movements which separate the cuticles; no stylet movement or pumping of the oesophageal valve was noted at this stage. As the moulted cuticle becomes clearly separated in the head region, longitudinal and rotary motion resumes within the egg. Occasional pumping of the median oesophageal valve is accompanied by extrusion of the stylet. About thirty hours after laying, the first moult is complete; the juvenile is about three times as long as the egg and movement is continuous until hatching one or two hours later.

FIGURE 27. Emryology of *Seinura demani* (see text for explanation).


40µ
A
B
C
D
E

Hatching: Movement continues with pressure being applied to the egg membranes by flexure of the cephalic region at each pole. Pumping of the oesophageal valve and extension of the stylet increases in frequency. Although on occasions the spear penetrates the egg shell in the process of sucking moisture from the media around the egg, the stylet action is not usually directed against the wall. Over the last four to five minutes before hatching the shell becomes elastic and the egg increases in size by 3-4μ . The juvenile emerges by stretching the wall until it ruptures, or by extending the wall and bursting it with a single jab of the stylet tip.

Post-hatch juveniles (n = 20): L = 220-350μ ; a = 19.4-24.0; b = 5.2-5.5; c = 4.3-5.3. Stylet about 11μ long, oesophageal gland lobe 50.0-80.2μ long. The genital primordium is 10.0-20.0μ long, consists of four cells at 55% to 65% of the body length and grows to about 60.00μ during the moulting period.

The nematodes begin feeding soon after they hatch. Most feed on the nearest cadaver which had been partially consumed by the parent at the time of laying, some were observed feeding on eggs of A. avenae, Plectus sp., and S. demani, others immobilised and killed prey many times their own size. Juveniles hatched in the absence of prey survived up to five days, but showed no further development unless prey were introduced. Feeding occurred even after four days starvation prior to the introduction of prey.

Pre-adult juveniles: Females (n = 10): L = 420-580μ ; a = 26.1-32.0; b = 7.9-10.0; c = 4.8-5.7.

Males (n = 10): L = 305-430μ ; a 26.3-29.6; b = 6.0-8.7; c = 5.5-8.0.

Early in this stage the genital primordium is

55-75 μ long, increasing in length by 70-100% in females and up to 60% in males by the time the pre-adult moult is completed. During the moult the vagina, uterus and spermatheca are differentiated, and in males sclerotisation of the spicules takes place.

Adult: Females (n = 10): L = 602-720 μ ; a = 28.4-34.0; b= 9.1-11.0; c = 5.3-7.3; V = 64.0-72.0%; G = 38.0-45.0%.

Males (n = 10): L = 390-490 μ ; a = 26.9-32.0; b = 7.1-8.4; c = 9.5-15.6; T = 82-87%; G = 25-36%.

Females: head offset, stylet knobbed, 16-18 μ long. Excretory pore in front of nerve ring, but adjacent to, or about 4 μ behind the posterior end of the oesophageal bulb. Ovary straight, oogonia in three rows at the widest part, reaching past the posterior end of the oesophageal gland lobe. No post-vulvular sac present.

Males: Smaller than females, the spikate posterior end of the tail set off by a marked constriction at about 70% of the distance from the tail tip to the anus. A post-anal pair of papillae about 16 μ behind the spicule tip, and one adanal pair opposite the rostrum.

Females copulate immediately after the final moult and the first egg is laid about five hours later. Virgin females do not lay eggs. During oviposition the vaginal orifice is distended about 3-4 μ , and the elastic nature of the egg shell is evidenced as the egg 'flows' through the narrow aperture. Well fed females laid one egg about every two and one-half hours.

Males and females develop at the same rate, and there was no apparent sex difference in longevity. Five males survived 11 to 13 days in chambers with

abundant prey while five females in the same conditions survived 10 to 14 days. Total generation time (egg to egg) at 20°C is four and one-half to five days.

MATING BEHAVIOUR: Up to five active male S. demani have been observed closely aligned to one pre-adult moulting female (Figure 26C). They repeatedly nuzzle the female as if attacking, but without extension of the stylet; the tail is coiled and uncoiled around the female body. The nudging and coiling is sometimes interspersed with brief periods of inactivity, or movement up to about 250μ away from the moulting individual but they repeatedly return to the sub-adult female locality and resume a hyper-active behaviour pattern until the moult is completed.

On casting the last juvenile cuticle, the adult female moves backward or forward while the male assumes the copulatory posture with its tail ventrally curled and the spicules projecting into the loop (Figure 26D). Copulation lasts up to 15 seconds. The successful male inserts the spicule tips into the vulva, forcibly injecting spermatozoa into the spermatheca. Active migration of spermatozoa was not observed. More than 200 spermatozoa have been counted in the spermatheca of young females. Remaining males continue their agitated behaviour pattern, and on a few occasions a second male was successful in mating before the female moved off in search of prey.

SEX ATTRACTION: Males did not attempt to follow individual females when placed on their tracks, nor were they attracted to cellophane barriers separating adult females from them.

No evidence for the presence of an attraction mechanism toward pre-adult moulting females transferred to sterile water agar was observed. Males exhibited random movement through the medium, and only on one occasion was a stable relationship with a pre-adult female established. However, males observed in culture chambers containing populations of prey and predator were attracted to pre-adult females. When males came to within 1.0-1.5mm of the moulting individual, movement becomes 'agitated' and is punctuated by frequent back-tracking, reversal, lateral probing of the head and occasional periods of inactivity. This pattern continued until the male moved out of the field of influence, or the female is located. Movement of males toward females is not direct, but is orientated toward the general vicinity by trial and error movement over localised areas. Contact is usually ensured once a male comes to within a distance of 400-500μ from the female, but exceptions occur. Nine out of 12 'activated' males located pre-adult moulting females.

In many instances several males are attracted to one female by the time the moult is completed. After copulation the female moves away; unsuccessful males do not attempt to follow, but remain in the vicinity of the cast cuticle maintaining an agitated state and assuming copulatory positions about other unsuccessful males and about themselves for up to 15 minutes. On one occasion four males did not attempt to track a female which became active before rupturing the juvenile cuticle and emerging.

Adult males were never observed in association with pre-adult moulting males or moulting post-hatch stage juveniles. Similarly, adult females were not seen

aggregating in the vicinity of pre-adult moulting females or pre-adult males.

Movement of adult males was not influenced by the presence of pre-adult moulting females separated from them by an air space of less than 1mm.

SEX RATIO: There was no significant difference in the ratio of males to females between the three treatments. The ratios ranged from 1:1.6 to 1:3.1. In treatment two, in which prey were not replaced, the total number of adults was depleted at harvest (29 and 40 adults for respective replicates) without significantly altering the sex ratio.

DISCUSSION

The developmental biology of Seinura species is characterised by an exceptional variability within the genus: S. tenuicaudata (de Man, 1895) Goodey, 1960, reproduces bisexually and undergoes four moults, the first within the egg, before reaching maturity (Hechler, 1963); S. steineri Hechler, 1965, is hermaphroditic and undergoes four moults (Hechler and Taylor, 1966a); S. oxura (Paesler, 1957) Goodey, 1960, is hermaphroditic with two moults within the egg and two post-hatch moults (Hechler and Taylor, 1966); S. celeris Hechler, 1965, and S. oliviera (Christie, 1939) Goodey, 1960, are bisexual and undergo one moult within the egg and two further moults after hatching (Hechler and Taylor, 1966a). S. demani follows the same pattern as S. celeris and S. oliviera. Nothing suggestive of a second moult within the egg was observed.

Hechler (1963) demonstrated feeding and reproduction of Seinura species on Ditylenchus dipsaci (Kuhn, 1857)

Filipjev, 1936, larvae of Hetorodera trifolii Goffart, 1944; Meloidogyne hapla Chitwood, 1949, and Neotylenchus lindfordi Hechler, 1962. Lindford and Oliviera (1937), reported that Seinura species fed on larvae of Meloidogyne species, on Pratylenchus pratensis (de Man, 1880) Filipjev, 1937 and Aphelenchoides parietinus (Bastian, 1965) Steiner, 1932, as well as Aphelenchus avenae. The prey list was extended in the present study to include bacteriophagous nematodes; further evidence for the non-selective predatory habits of Seinura species.

Feeding of S. demani involved secretion of fluid from the dorsal oesophageal gland into the prey as described by Lindford (1937a), and Hechler (1963). No variation from this pattern was observed.

Availability of prey did not influence the sex ratio of S. demani significantly, but the total number of adults in cultures with depleted prey was markedly reduced. Under conditions of limited prey, cannibalism between juvenile and adult S. demani was frequently observed. Similarly, in the absence of prey or a fresh cadaver, post-hatch juveniles attacked eggs in the immediate vicinity. As eggs are produced singly, or in groups of two or three, predation by the first juvenile to hatch could have marked repercussions on the rate of population development. Post-hatch juveniles require to feed soon after hatching for development to continue. Hence juveniles hatched from single eggs would have less chance of survival, as would slower developing eggs within groups. Cannibalistic feeding between adults and juveniles was most in evidence on inactive moulting stages, which means that all animals must pass through a period of greater susceptibility in the course of their development.

In her study on S. tenuicaudata, Hechler (1963) found that the ratio of males to females ranged between 1:1.2 and 1:2.5 in cultures of different ages and cultures under various conditions. Indiscriminate cannibalistic feeding was reported in the absence of prey species. Hechler and Taylor (1966a)found variations of 1.78 to 7.4 females per male for S. celeris and 4.0 to 20.0 for S. oliviera but do not comment on the abundance of prey when these results were recorded. It seems that in Seinura species population development is limited under conditions of reduced prey abundance by cannibalism at various stages in the life-history.

The presence of a sex pherome produced by a pre-adult moulting female at a stationary locus greatly increases the chances of a successful mating in S. demani. Such a mechanism must be maximally advantageous too, when seasonal 'blooms' of prey species occur as fertilisation of females on completion of the final moult ensures that the generation interval is kept to a minimum, enabling the predator to increase numbers rapidly.

Greet (1964) suggested that the sexes of Panagrolaimus rigidus were brought together by water soluble chemical attractants of small molecular size, and that different specific substances were produced by each sex. Jones (1966) showed that chemical attraction was involved in the location of Pelodera teres females by males, but did not demonstrate mutual attraction between the sexes. Again the use of a dializing membrane indicated that the substance was of small molecular size. Similar results were obtained by Chin and Taylor (1969) for Cylindrocorpus longistoma Stefanski, 1922, and

Cylindrocorpus curzii (Goodey, 1935) Goodey, 1939, who considered the attractants to be species specific as well as sex specific. Green (1966) concluded that the attraction factor produced by sedentary female Heterodera schactii and H. rostochiensis was chemical; males were orientated to the stimulus from at least 5mm and were still attracted 20 hours after the females were removed. Later, the same author (Green, 1967) showed that gustatory rather than olfactory perception of the stimulus was involved.

S. demani males were attracted to moulting females in agar over a range up to 1.5mm, and after the exit of females, males were retained at the site of the moult for only short periods. Although the sex pherome does appear to be chemical as suggested by previous workers (Greet, 1964; Green, 1966; Jones, 1966; Chin and Taylor, 1969), it is probably of larger molecular size than the attractants involved in these associations and appears to be broken down readily. Males were attracted to females at various stages of the moult. They were retained in close association with the pre-adult female for long periods before the moult was completed. As the factor breaks down rapidly it is probably produced continuously throughout the moult to maintain the gradients in the immediate environment. The failure of pre-adult females to attract males four hours after rinsing in sterile water does not preclude this suggestion, but may have been due to the low diffusion rates of the attraction chemical and the disturbance factor involved in placing the animals on a fresh medium. If the attractant is produced continuously, then build-up in the range of the stimulus over the moulting period could be expected. The fact that most single male, pre-adult moulting female associations involved females in the middle of a moult,

and multiple male, moulting female associations usually involved late moulting stage females is indicative of such a mechanism.

Green (1966) concluded that males of H. schactii and H. rostochiensis exhibited klinokinetic aggregation behaviour and klinotaxic orientation. The trial and error pattern of movement initially exhibited by male S. demani and the final location of the female suggest that a similar mechanism is involved. The same author (Green, 1966), suggested that at high concentrations of the attractant, receptors could become fatigued with a decrease in the acuity of perception affecting the orientation response. This could explain the occasional circling of pre-adult moulting females by males which did not lead to final contact. Similarly, as suggested by Green (1966), fatigue of receptors may explain why males periodically move short distances from females before reversing and returning to the immediate vicinity. Anderson and Darling (1964b) considered that the amphids may be the sensory organism involved in the orientation of male Ditylenchus destructor to their females. The continual lateral probing of the head of male S. demani indicate that the sensory receptors are located in the head region.

Copulation between adult male and female S. demani was observed but appeared to result from random encounters. There was no mutual attraction or repulsion between the sexes either as adults or pre-adults.

6.7. DELADENUS DURUS (COBB, 1922) THORNE, 1941.

INTRODUCTION

Thorne (1961), Goodey (1963) and Clark (1964) considered that species were probably of mycophagous habit. This was not substantiated until Bedding (1967) reported observations on two new species of Deladenus which he subsequently described as D. wilsoni and D. siridicola (Bedding, 1968). The life-history of these species involved female dimorphism associated with parasitic and free-living cycles (Bedding, 1967). The free-living mycetophagous form was typical of the genus Deladenus (Neotylenchidae) and the parasitic form, which reproduced in the haemocoel of siricid wood wasps was typical of the Allantonematidae (Bedding, 1967, 1968). Infective females of the parasitic stage were produced along with females of the free-living form, on cultures of the fungus Amylostereum chailletii (Fr.) Boidin.

In the present study Deladenus durus (Cobb, 1922) Thorne, 1941, was isolated from the rhizosphere of fescue tussock plants. Observations were made to investigate the biology of the nematode as a mycetophagous free-liver with particular interest in the possible occurrence of female dimorphism in laboratory cultures.

METHOD

Host range tests were carried out on U. atrum, F. oxysporum, P. setosa, A. tenuis, P. herbarum, R. solani, Z. moelleri, G. roseum, P. cactorum, and M. alpina, cultured on 1.0% water agar in glass ring chambers. Gravid females were placed on the cultures and incubated at 20 C. A fungus species was regarded as a suitable host

if a generation was completed.

Life-history and feeding studies were made on nematodes raised on U. atrum on 1.0% water agar at 20 C. Observations were made on nematodes cultured in glass ring chambers and on individual animals placed on the surface of the media and viewed through a cover glass which was carefully lowered over them.

RESULTS

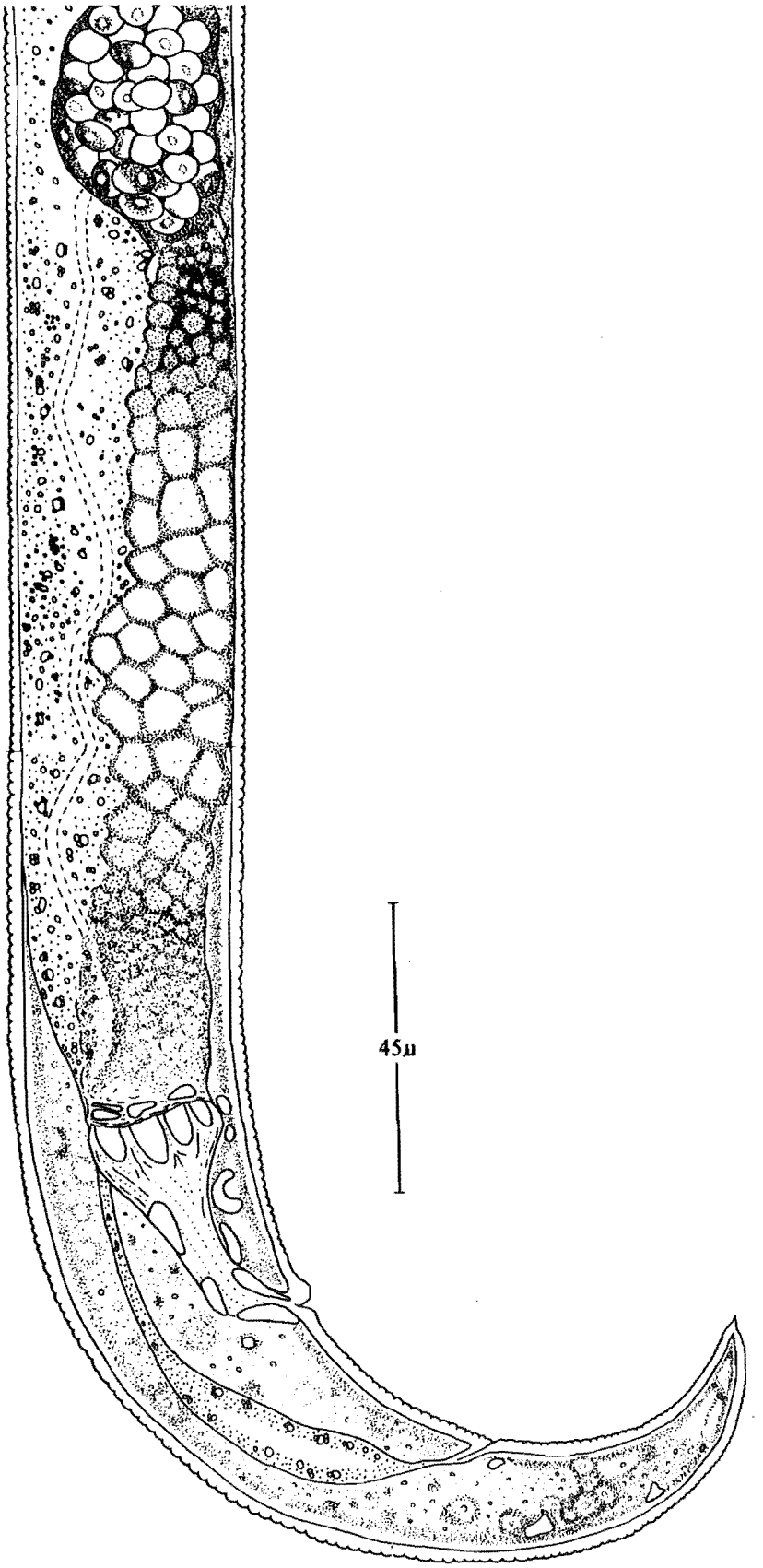
MORPHOLOGY: Measurements: Specimens mounted in glycerine. Females (4): L = 765-902μ ; a = 42.6-48.1; b = 8.6-10.3; c = 17.3-23.0; V = 91.6- 95.2%. Males (4): L = 550-840μ ; a = 44.1-60.0; b = 6.8-8.7; c = 16.6-21.0; T = 90.0-95.2%. Juveniles (4): L = 445-594μ ; a = 39.2-47.5; b = 6.6-7.0; c = 17.5-18.2

Specimens mounted in temporary water mounts Females (5): L = 801-1083μ ; a = 33.5-39.0; b = 8.1-9.8; c = 18.6-23.5; V = 88-94%.

Body tapering anteriorly, cuticle marked by transverse striae about 1.2-1.5μ apart. Lateral fields consist of seven insicures delimiting six raised elements. Stylet 8-9μ long with well developed basal knobs. Vulva protruding, vagina muscular, heavily sclerotised terminating in an expanded fimbriate neck with muscular claw-like extensions (Figure 28). Uterus lined with large cells, granular in appearance, usually three high. Spermatheca posterior to the oviduct, slightly more than a body-width in length, packed with spermatozoa about 5μ in diameter. Tails of males and females sharply tapered, termini varying from acute to sub-ventrally mucronate.

HOST RANGE: D. durus reproduced on all of the fungi tested.

FIGURE 28. Morphology of tail region of a female Deladenus durus.


45μ

FEEDING: The eelworm moves sinuously through the medium, and when its lips touch a hyphal strand it usually assumes position characterised by curvature of the oesophageal region (Figure 29A) and probing of the stylet begins until penetration is achieved a few seconds later. Within five minutes a hyaline vacuole appears at the base of the stylet. Granular material was observed oscillating in the vacuole, but no movement toward the stylet base was noted, nor was material seen moving to or from the stylet tip inserted in the hyphal cell. The valvular apparatus in the corpus of the oesophagus of Deladenus species is very much reduced. During feeding vibrations of the corpus occur, and although there was no evidence of active pumping in the vicinity of the rudimentary valve plates a slight vibration was observed throughout the oesophageal lumen. Irregular movements occurred at the anterior end of the intestine, but they were difficult to observe accurately because of the overlapping dorsal oesophageal gland, the presence of the nerve ring and granular contents of the intestine. Feeding continued for up to three hours at one site, and migration between feeding periods was usually restricted to a distance of a few microns. No obvious modifications to parasitised hyphae occurred during feeding, but cells of septate mycelia appeared more hyaline a few hours after feeding was terminated.

REPRODUCTION: Both sexes are necessary for reproduction. Active male nematodes were observed in close association with pre-adult moulting females, suggesting a similar mechanism to that demonstrated for Seinura demani (section 66).

Copulation: Copulation occurs soon after the moult is completed. Males exhibit periods of agitated activity

FIGURE 29. Feeding, copulation and egg laying of Deladenus durus. (f = female; m = male; oo = oocyte; ov = ovary; sp = spermatheca= ut = uterus; sh d = shell deposition.

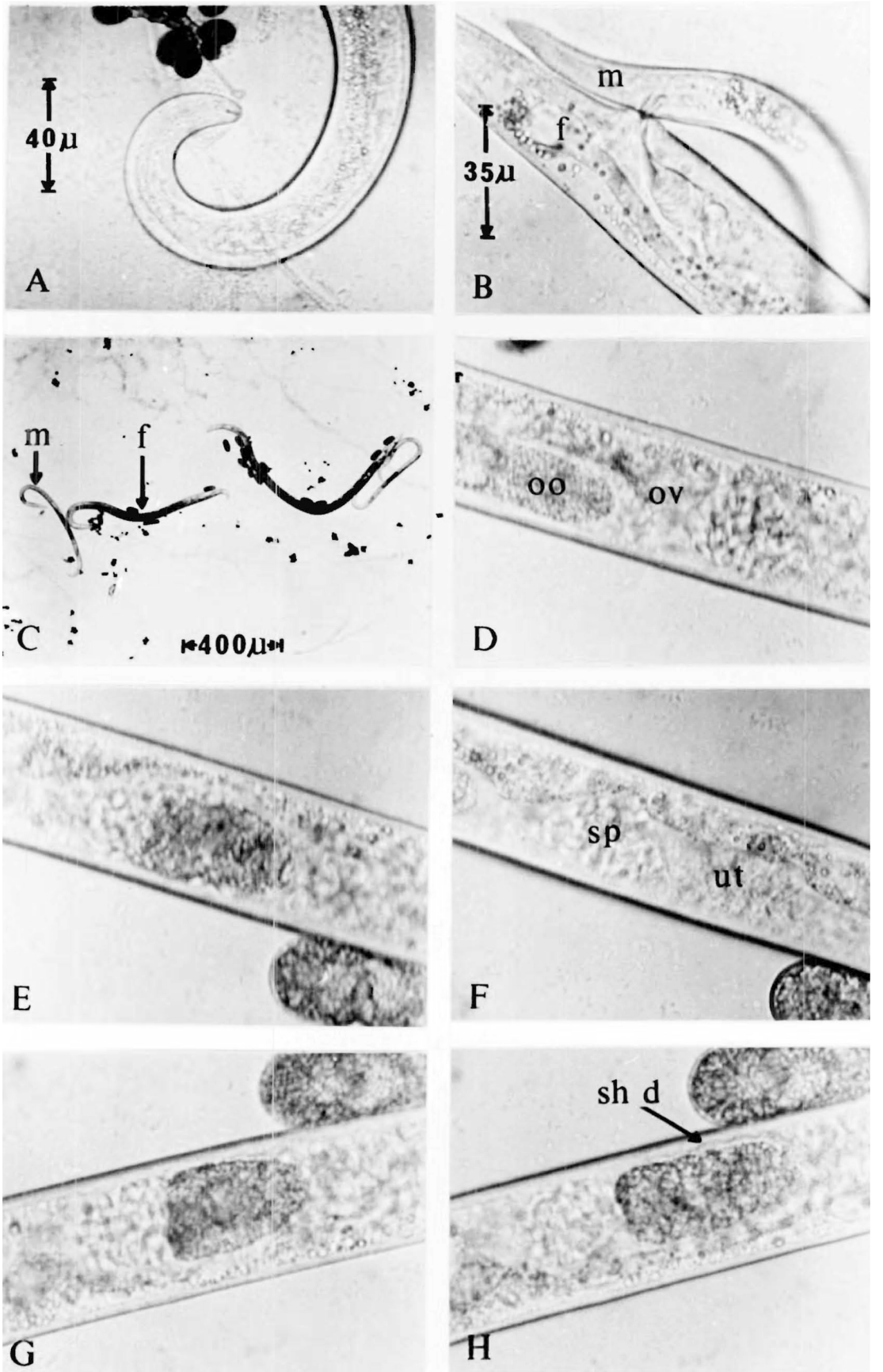

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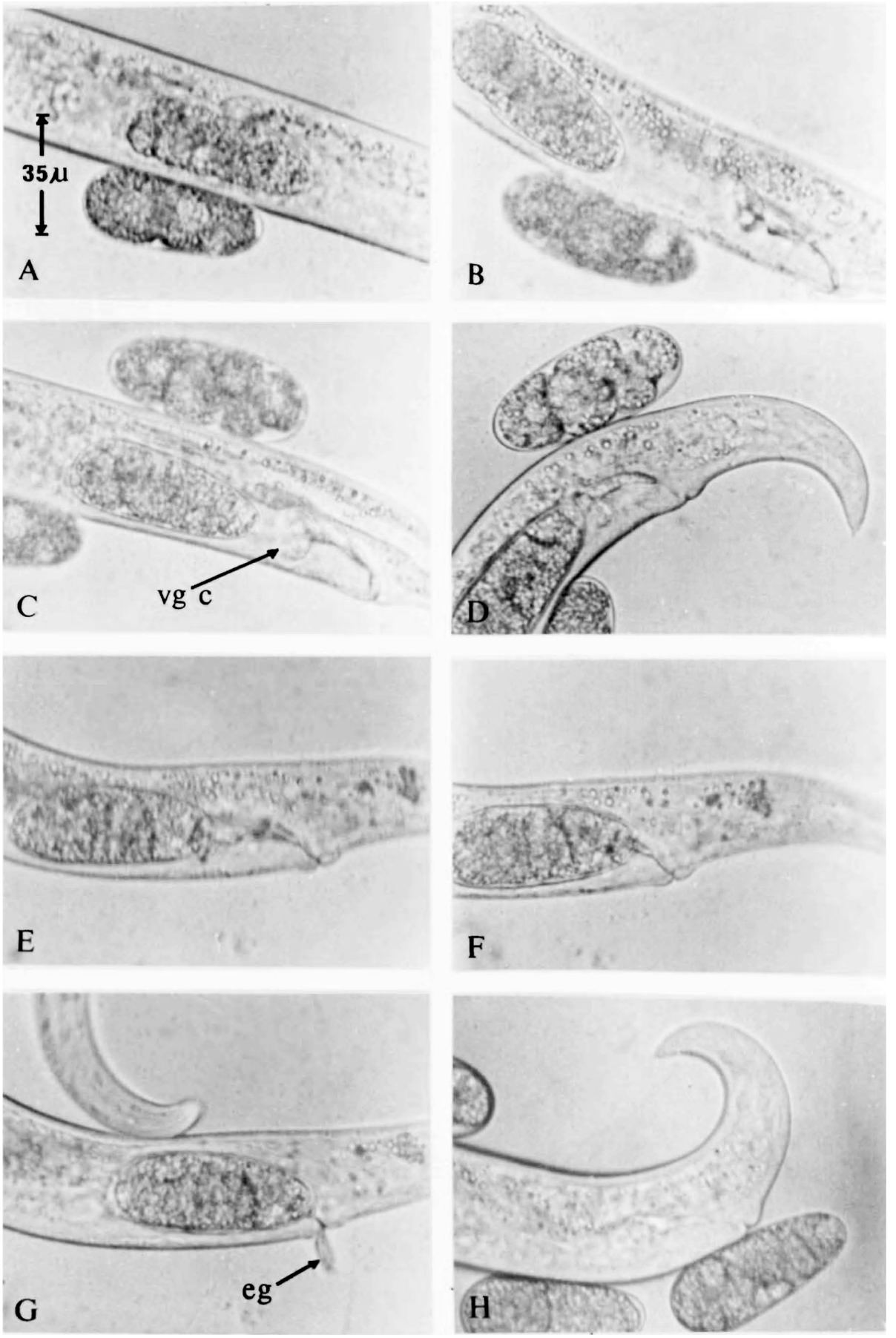
FIGURE 30. Egg laying of Deladenus durus continued.
(vg c = vaginal clasp; eg = egg).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prior to copulation, nudging and projecting their stylet against the female body. The female continues feeding with no apparent acknowledgement of the presence of the males although periodic contractions of the vulva were noted.

During copulation males align their tail parallel to the female vulva region and move backward and forward while extruding the spicule tips. On contact with the vulva the spicules are inserted and the caudal alae lie along the body of the female (Figure 29B). Insemination occurs immediately causing the anterior vaginal region to become swollen with sperm cells (Figure 29B). Copulation lasted 10-20 seconds (five observations). The amoeboid sperm cells move slowly up the uterus, taking approximately 25 minutes to clear the vagina, finally aggregating in the spermatheca immediately posterior to the oviduct (Figure 29D and F).

Males continue to feed in the vicinity of the females after copulation (Figure 29C), or sometimes move off and associate with another female. Copulation was observed on three occasions during the life of one female.

Fertilisation: The oocyte moves slowly forward toward the oviduct (Figure 29D), and assumes a dumb-bell shape as it flows through the constriction. Compaction of the oocyte takes place just forward of the spermatheca (Figure 29E). Sperm cells become active for 1-2 minutes, and up to four cells have been seen to move into the granular oocyte. The egg protoplasm contracts as it moves into the uterus proper.

Shell formation: About eight minutes after penetration by the sperm, the first evidence of shell formation becomes apparent as a granular deposition about the periphery of the egg (Figure 29G and H). At

this time the large uterine wall cells posterior to the spermatheca (Figure 29F) appear to become more granular and may play a role in development of the shell membranes. The shell is completed as the egg continues its slow movement through the uterus toward the vagina (Figure 30A-C).

Egg laying: No muscular contractions were observed until the egg entered the clasp-like opening of the vagina. At this point (Figure 30 D and E) flexing of the anal tract in a posterior direction occurs accompanied by spasms of muscular movement above the vulva. Further movement into the vagina is assisted by ventral curvature of the tail. The curvature is slowly reversed dorsally with a final flick of the tail and contraction of muscles around the vulval plates forcing the egg through the vulva (Figure 30F-H). The elasticity of the shell is evidenced as the egg moves through the narrow aperture, beginning as a bubble-like extrusion (Figure 30G) which increases in size until the egg is completely expelled. The vulval lips part by about 3-4μ during expulsion. Feeding is not interrupted during laying.

Embryonic development: Eggs are oval in shape, measuring 54-60μ x 22-26μ slightly narrower at the end which is first expelled. The eggs frequently appear to be segmented at laying (Figure 30H), but reconstitute to form a homogeneous body within 15 minutes of decomposition.

The first division occurs 1-3 hours after laying and results in two slightly unequal blastomeres. The smaller cell divides after a further 2-3 hours, division of the larger cell following 1-2 hours after this. Most eggs reach the five blastomere stage within

12 hours of laying. Further divisions were not followed.

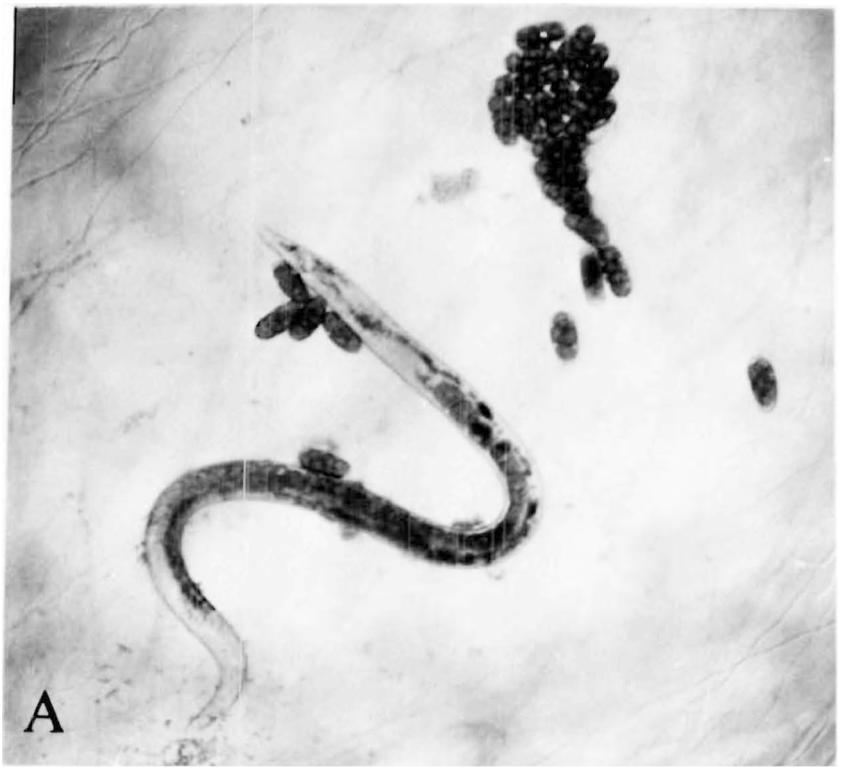
Movement of the embryonic nematode begins after 50-60 hours at which stage the embryo is about $1\frac{1}{2}$ times as long as the egg. In the next 24 hours the juvenile doubles its length, and at 90-100 hours a faintly defined stylet is apparent: spear knobs are poorly delimited and the shaft did not appear to be sclerotised.

Post-embryonic development: The first moult occurs within the egg about 110-120 hours from laying. Separation of the first-stage cuticle is obvious in the lip region and during the moult a second stylet with well defined shaft and knobs is formed. The intensity of movement within the egg increases after the moult, and stylet probes are directed against the egg wall. About six hours before hatching distortion of the egg shell is apparent. For the next few hours the juvenile increases in size and distortion of the egg becomes more pronounced. Hatching finally occurs $5\frac{1}{2}$-6 days after the egg was laid. Rupture of the cuticle is achieved by constant probing of the stylet against the poles of the egg accompanied by flexure of the tail and cephalic regions.

The juvenile undergoes two further moults before entering the fourth moult about 6-7 days after hatching. The quiescent period lasts 24-36 hours. Adult females begin laying within 12 hours after copulation. Three to five eggs are laid on the first day but the frequency increases to one every two hours by the third day. The total number of eggs laid ranged from 50-200 (six females). As migration during feeding and laying was restricted, the eggs were deposited in large groups (Figure 31A and B). No evidence of female dimorphism was observed.

The minimum generation time (egg to egg) was 13-15 days at 20°C.

FIGURE 31. Eggs laid in clusters by *Deladenus durus*.
A) Gravid female depositing eggs in 'clusters'. B) 'Clusters' of eggs at various stages of development.


A


B

DISCUSSION

Morphological characters of the Broken River specimens agree with the descriptions of D. durus reported by Cobb (1922), and Thorne (1941) with the exception of the shape of the tail terminus. Cobb (1922) described the tail as straight conoid, acute, convex-conoid, unarmed, and symmetrical, but Thorne (1941), illustrated variations in termini of females which included bluntly rounded, acute, bifurcate to quadrifurcate forms. The termini of Broken River specimens ranged from acute to sub-ventrally mucronate. Variability in tail shape was also noted by Das (1964).

An undescribed feature of the Broken River specimens was the presence of a heavily sclerotised vagina opening into the uterus through an expanded muscular claw-like orifice. Cobb (1922) referred to the 'medium sized, muscular, somewhat cutinised vagina' as extending inwards and 'obliquely forward about half way across the body', and Thorne (1941), mentioned a heavily sclerotised vagina leading in and forward to the muscular uterus, but neither worker noted the presence of a claw-like vaginal opening. In animals which had been processed to glycerine the musculature tended to collapse, obscuring the shape of the neck which may account for the failure of Cobb and Thorne to comment on its structure. Muscular collapse is further evidenced by the high values of 'a' for processed animals relative to the values for live specimens.

D. durus was cultured on a wide range of fungi, indicating that its distribution is unlikely to be governed by host specificity.

In view of Beddings research (Bedding, 1967, 1968), and the previous association of D. durus with galls of

the chestnut oak Quercus prinus L., (Cobb, 1922), and under the dead bark of cottonwood, Populas fremonti S. Watts., (Thorne, 1941), it seems reasonable to suggest that D. durus may also exhibit an entomophagous stage in its life-history. However, dimorphism was not observed in populations raised under culture conditions. Three interpretations seem possible: that the Broken River isolate of D. durus has never possessed such an entomophagus stage in its life-history; that such a stage has been lost over several generations of the mycetophagous form; or that the free living stage requires a stimulus to initiate the development of the parasitic stage. Symbiotic associations between certain basidiomycete fungi are well established and it is suggested that siricid larvae require the presence of a specific fungus for completion of development (Gilmour, 1965). The fungus A. chaelletii is symbiotically associated with siricids (Bedding, 1968) and hence may provide a stimulus for the induction of dimorphism in cultures of D. wilsoni and D. siridicola. A similar mechanism may be necessary for dimorphism to occur in cultures of D. durus.

6.8 APORCELAIMELLUS PARAAMYLOVORUS N. SP.

INTRODUCTION

Aporcelaimellus paraamylovorus n. sp. was characterised as a miscellaneous feeder in Chapter 3. Aporcelaimellus Heyns, 1965, species were previously considered to be predominantly vegetarian (Heyns, 1965). The biology of A. paraamylovorus was considered in detail as

an example of a miscellaneous feeding nematode.

METHOD

Host range: In general feeding trials (Chapter 3), A. paraamylovorus was observed feeding on algae, moss protonemata, nematodes, nematode eggs, enchytraeids, and rotifers, but the animals did not survive on bacteria, fungi or seedling roots. Populations of the nematode were established on Haematococcus in soil extract agar in the absence of other animals, but bacteria and fungi were present. Nematodes from these populations were used to test the suitability of a number of species of algae representing a range of cell shapes and sizes, as food organisms for A. paraamylovorus.

Cultures of Hormidium nitens Menegh. (335/3)*, Chlorococcum sp., Dictyococcus engadinensis (Chad. et Kol) Vischer (221/3)*, Dactylococcus sp., Pleurochloris magna Petersen (860/2)*, Protosiphon sp., Chlamydomonas sp., and Chlorella vulgaris Beij., were established in 250 ml flasks containing about 100ml of Czurda's medium. The flasks were incubated at 18°C under artificial light until a bloom was established. Each stock culture was harvested by suspending the cells in sterile water. The suspension were concentrated by settling, and 1.0ml of the concentrate was resuspended in 6.0ml of cooled 1.5% soil extract agar in 4.5cm diameter Petri plates. Two plates were prepared for each species of alga together with two additional plates containing a mixed flora dominated by Haematococcus. Three adult A. paraamylovorus

* From the culture collection of algae and protozoa (C.C.A.P.), Cambridge, U.K., dated September, 1958.

were placed in one plate of each pair, and three juveniles on the other. The cultures were incubated at room temperature under natural light. Every third day, sterile water was added to the surface of the media to minimise desiccation.

RESULTS

DESCRIPTION: (see Figure 32A-H).

Holotype female: L = 1.48mm; a = 26.3; b = 4.1; c = 55.1; V = 55%; G_1 = 25.6%; G_2 = 18.6%

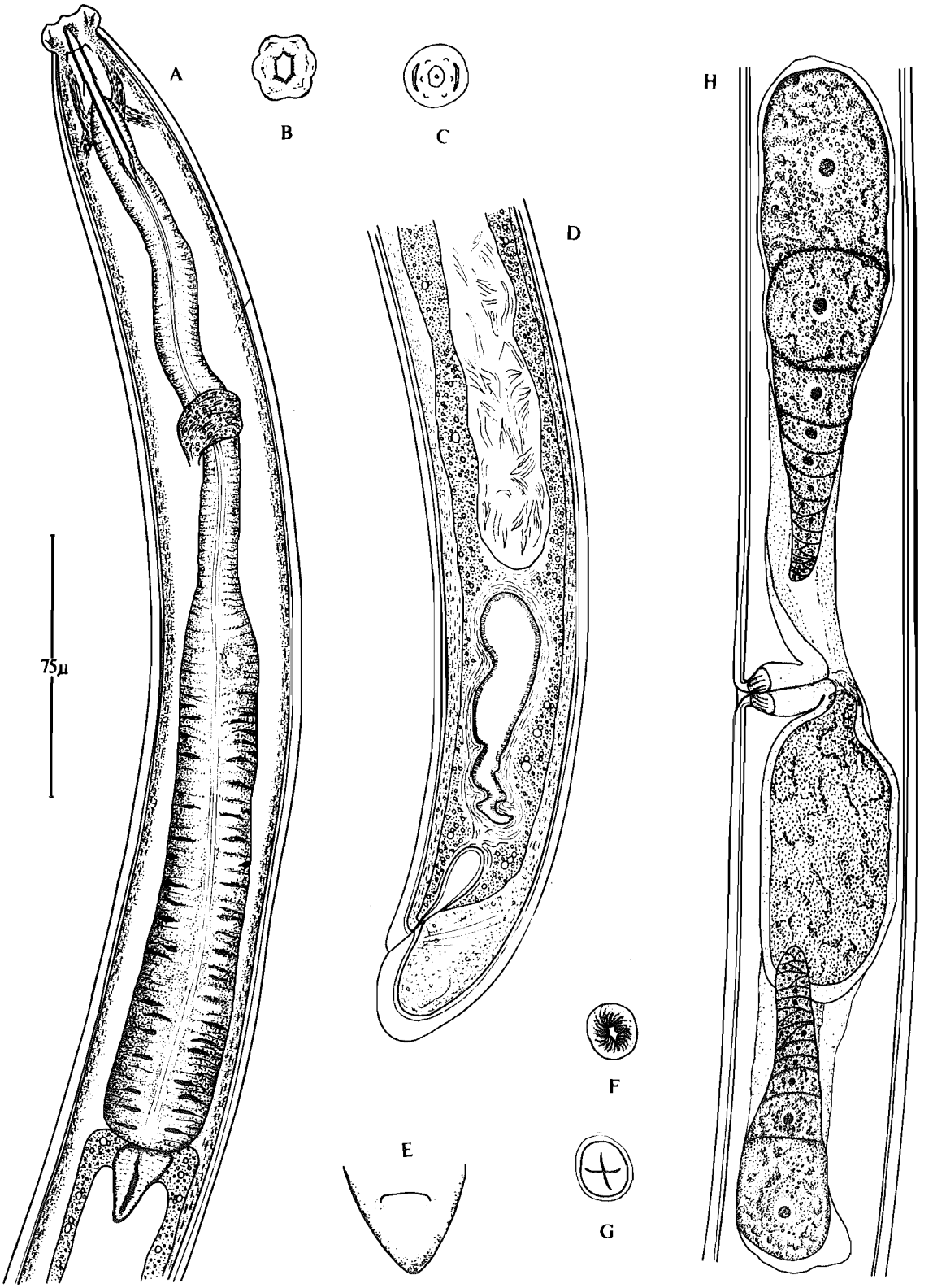
Paratypes (n = 19 females): L = 1.30-1.58mm (1.43); a = 23.2-32.4 (26.0); b = 3.84-4.2 (4.0); c = 50.7-68.3 (58.5); V = 48.3-57.0% (54.1); G_1 = 14.5-28.3% (20.5); G_2 = 13.7-29.2% (19.3).

Males unknown.

Body cylindroid, tapering slightly anteriad and posteriad from about the level of the vulva, straight to gently curved ventrally when relaxed by gentle heat. Cuticle and sub-cuticle appear smooth. Thick muscle layer beneath the cuticle.

Lip region set off by a marked constriction, with six distinct lips (Figure 32A-B). Diameter of head about 19μ, diameter between lateral lips about 17μ. Inner ring of six labial papillae and an outer ring of visible in en face view. Oral aperture hexagonal, posterior portion of the stoma oval to hexagonal (Figure 32B-C). Amphid apertures large, 9-10μ x 1.0μ, outwardly convex. In lateral view amphids are cyathiform with the sensilla pouch about 10μ behind the odontostyle base. Odontostyle 22-24μ long, 4μ wide, tip rounded, aperture about one-third of the total odontostyle length. Guiding ring simple, anterior margin smooth.

FIGURE 32. Aporcelaimellus paraamylovorus n. sp. A) Oesophageal region. B) En face view of lip region. C) En face view through amphids. D) Anal region, including Pre-rectum and posterior intestine. E) Ventral view of anus. G) Optical section through vagina. H) Vagina and gonads in lateral view.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Anterior portion of the oesophagus one-third of a body-width wide, nerve ring at about 60% of its length, excretory pore about one-half body-width anterior to nerve ring (Figure 32A) Posterior bulb about 50% of the total length of the oesophagus, about one-half body-width wide at the base. Oesophageal lumen cuticularised throughout. One oesophageal gland nucleus, dorsal, anteriad, difficult to see in lateral view. Oesophago-intestinal disc absent, oesophago-intestinal valve triangular (Figure 32A).

The walls of the oesophagus contain scattered granules, particularly marked at the oesophago-intestinal junction and interspersed with large prominently nucleated cells along its length. In specimens devoid of food material in the gut, the intestinal lumen appears folded throughout, but particularly so in the posterior half (Figure 32D). Rectum slightly longer than one anal-body-width, with a muscular sphinctor at the junction of the pre-rectum. Pre-rectum about twice as long as anal-body-width, walls muscular, lined with micro-villi. Anus about 15μ wide, a transverse slit, outer extremities directed posteriad (Figure 32E). Tail less than one anal-body-width long, convex to conoid, bluntly rounded.

Lateral cords about one-sixth of a body-width wide, contain 45-50 pores, of which eight to 10 occur in the neck, 20-25 in the dorsal and ventral cords.

Vulva pore-like to longitudinal (Figure 32F), with cuticularised lips. Vaginal lumen cross-like (Figure 32G); vagina extends up to 50% of the body width. Gonads paired opposed, reflexed at 50-60% of their length (Figure 32H).

Juveniles similar in general morphology to adults. Juvenile stages can be distinguished by the length of the primary and secondary odontostyles. Measurements of

odontostyle length for the four juvenile stages (from animals raised in the laboratory): First stage juveniles, primary odontostyle, 7.0-8.5μ , secondary odontostyle 10.0-11.0μ ; second stage juveniles, primary odontostyle, 10-0-11.0μ , secondary odontostyle, 13.5-15.0μ ; third stage juveniles, primary odontostyle, 13.5-15.0μ , secondary odontostyle, 16.0-19.0μ ; fourth stage juveniles, primary odontostyle, 17.5-19.0μ , secondary odontostyle, 22.5-24.0μ ; adult, primary odontostyle only, 22.5-24.5μ .

Type locality: From soil around roots of Festuca novae-zelandiae at Broken River (map reference N.Z.M.S. 1, Sheet S66); altitude, 720 m.s.l.

Diagnosis: Aporcelaimellus paraamylovorus is close to A. amylovorus, but differs in size (L = 2.00mm for A. amylovorus; L = 1.30-1.58mm for A. paraamylovorus), the shorter odontostyle aperture (one-half in A. amylovorus; one-third in A. paraamylovorus), the presence of an excretory pore, and the presence of pores in the dorsal, ventral and lateral cords. It is characterised by its small size, short tail, large amphid apertures, pore-like to longitudinal vulva, slit-like anus with extremeties directed posteriad, the presence of an excretory pore and the folded nature of the intestine lumen.

HOST RANGE: Large populations of A. paraamylovorus became established on Protosiphon sp., and Haematococcus sp., after three months. Some active animals were observed in cultures of Chlamydomonas sp., C. vulgaris, P. magna, Dactylococcus sp., and H. nitino after six weeks, but they were not in evidence after three months. A relationship between feeding success and the size of the algae cells is indicated (Table 18).

TABLE 18: Suitability and dimensions of algae tested as food organisms for A. paraamylovorus.

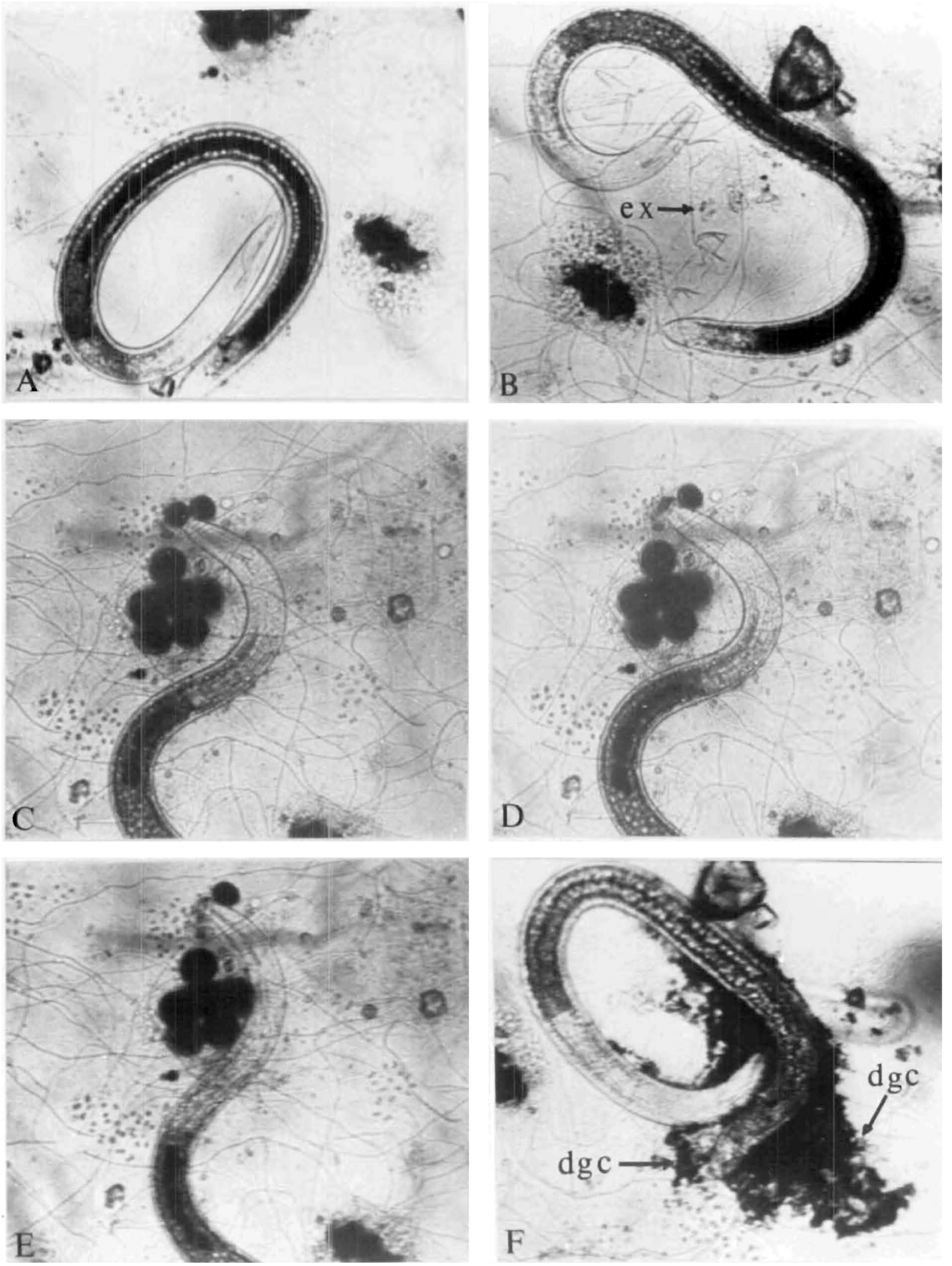
Food organism	Cell shape	Cell Dimensions (μ) 50 measured (mean)	Suitability
Haematococcus sp.	Spherical	15-45 (27)	+
Protosiphon sp.	Spherical	6-30 (19)	+
Pleurochloris magna	Spherical	4-9 (7)	-
Dictyococcus engadinensis	Spherical	4-12 (8)	-
Chlorococcum sp.	Spherical	3-18 (8)	-
Chlaymydomonas sp.	Spherical	5-18 (9)	-
Chlorella vulgaris	Spherical	4-9 (6)	-
Dactylococcus sp.	Spindle	3-4 x 14-25	-
Hormidium nitens	Rod (some filaments)	3-4 x 5-10	-

PENETRATION AND FEEDING: Protrusion of the odontostyle follows lip contact with a solid body in the media. The walls of the host cell are indented as the odontostyle is thrust against them and the first incision is made. Successive jabs sometimes accompanied by twisting of the head force the tip well into the parasitised cell. Ingestion does not occur until the odontostyle is inserted into the host as far as the parallel sides of the shaft. Forward and backward movements of the base of the oesophagus associated with contraction of the oesophageal valve muscles accompany pumping of the oesophageal valve lumen as ingestion begins. The movement is most pronounced in the posterior one-third of the valve. The contents of the parasitised cell are drawn through the orifice of the odontostyle, into the oesophagus, and can be seen passing into the intestine. Algae cells are completely denuded of their cell contents within seconds of the onset of ingestion (Figure 33C-E). Nematodes often have difficulty in removing the collapsed cell wall of algae and nematode eggs from about the lips on the completion of ingestion, even after the odontostyle was withdrawn. Violent sideways movement of the head and rapid reversal of direction were sometimes necessary to dislodge the cell remains.

Under the conditions in the agar medium, attempts by A. paraamylovorus to penetrate small alga cells are frequently unsuccessful; cells of less than about 10 μ diameter are usually brushed aside as the nematode pushes the odontostyle against the wall.

LIFE-HISTORY: Development within the egg: Eggs are oval, sculptured, and measure 87.2-92.3 μ x 41.5-44.5 μ (10 observations) at laying. The first division occurs after 10-15 hours, resulting in two blastomeres of about the

FIGURE 33. Feeding and defaecation activities of *A. paraamylovorus*.
A) Late moulting stage. B) Moult completed (ex = exuviae). C-E) Ingestion of the contents of a *Haematococcus* cell. F) Defaecation of solid material (dgc = defaecated gut contents).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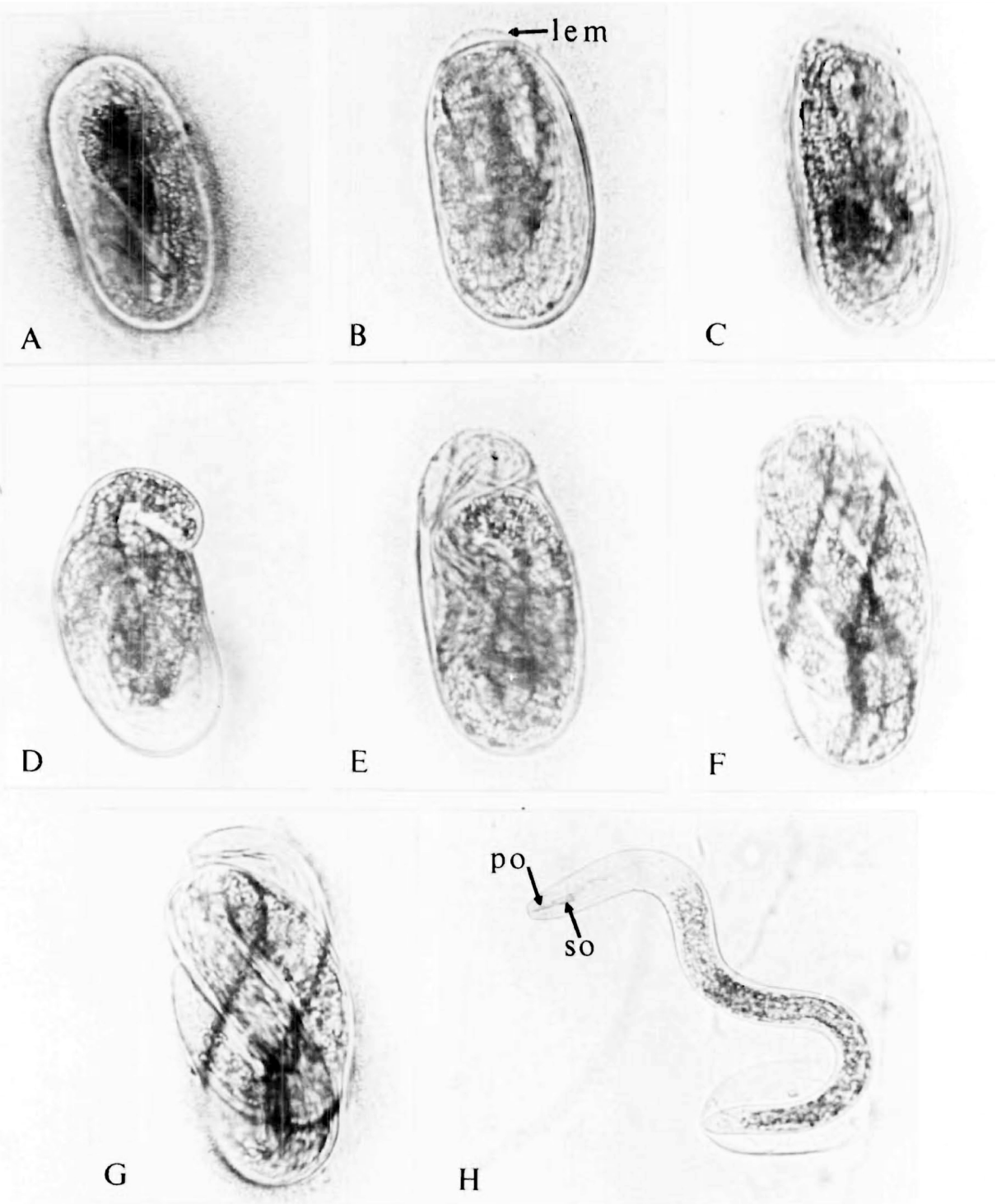
dgc

same size. About 24 hours from laying, four blastomeres are present, and approximately nine hours later the eight cell stage is reached. Subsequent divisions follow rapidly and the gastrula is reached three to four days from laying. After a further 24-48 hours the tadpole stage is apparent and movement within the egg begins. Six to seven days from laying the tip of the primary odontostyle is visible, but there is no evidence of the shaft; movement of the vermiform juvenile is almost continuous. Over the next 24-48 hours, the tip of the secondary spear becomes apparent, and the shaft of the primary spear is laid down. The juvenile is about three and one-half times as long as the egg. Longitudinal and rotary movement, interspersed with periods of inactivity, continue for 20-30 hours, during which development of the secondary odontostyle is completed (Figure 34A). The size of the juvenile increases to about four times the egg length. Pumping of the oesophageal valve is frequent, but movement of the stylet was not seen. Activity slows down and becomes more deliberate during the following few hours, pumping occurs periodically and some slight movement of the odontostyle occurs. About 230-250 hours after laying the outermost egg membrane lifts to form a bubble at the end next to the head of the juvenile (Figure 34B-C). The pliability of the inner membrane directly beneath the bubble is apparent as the nematode pushes against it. Pressure applied by flexure and rotation of the body during the ensuing 15 minutes distends the bubble further (Figure 34D-E). Protrusion of the primary odontostyle and pumping of the oesophageal valve increases in frequency and gradually the entire shell becomes pliable (Figure 34F-G). During the periods of pumping, liquid material within the egg flows toward the odontostyle orifice. From the time

FIGURE 34. Hatching of A. paraamylovorus.
A) Juvenile development within the egg is completed. B-C) Outer egg membrane lifting (lem = lifting egg membrane). D-E) Juvenile pushes into the lifted area. F-G) Pliability of the entire egg shell increases. H) Emerging first stage juvenile (po = primary odontostyle; so = secondary odontostyle).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separation of the egg membrane occurs to hatching, the egg increases in length by about 20% and in width by approximately 10%. Hatching is finally achieved about 10-12 days after laying by stretching the egg shell until it ruptures (Figure 34G-H). At the time of hatching, the secondary odontostyle lies in a position immediately posterior to the shaft of the primary odontostyle (Figure 34H).

Post-embyonic development: A. paraamylovorus undergoes four moults before reaching adulthood. The generation time in soil extract agar cultures of Haematococcus at 18±2°C is 95-130 days. Under these conditions, the first stage required 14-18 days for completion; the second stage, 16-23 days; the third stage, 26-34 days, and the fourth stage, 25-39 days.

The time for development of each stage appears to depend upon the availability of food and the condition of the medium in which the animal is cultured. Some pre-adult juveniles were able to complete their development in fresh cultures after existing for two to three months in an inactive form in cultures which were several months old and maintained in a relatively dehydrated condition.

Juveniles typically resemble adults except in size, the absence of reproductive organs, and in the number of odontostyles present. All juvenile stages possess two odontostyles except for a short period during each moult when three are present. The primary or functional odontostyle for any one stage is located in the mouth; the secondary or spare odontostyle is located in the musculature of the oesophagus. Adults possess a primary odontostyle only.

Measurements of juvenile stages: First stage juveniles (n =10): L = 375-525μ (451); a = 17.7-22.1 (19.7); b = 3.4-3.8 (3.6); c = 32.5 -40.4 (35.9); primary odontostyle = 7.0-8.5μ (7.7); secondary odontostyle = 10.0-11.0μ (10.4).

Second stage juveniles (n = 10): L = 540-710μ (603); a = 19.7-22.7 (21.2); b = 2.4-3.0 (2.9); c = 36.2-45.0 (40.9); primary odontostyle = 10.0-11.0μ (10.4); secondary odontostyle = 13.5-15.0μ (14.1).

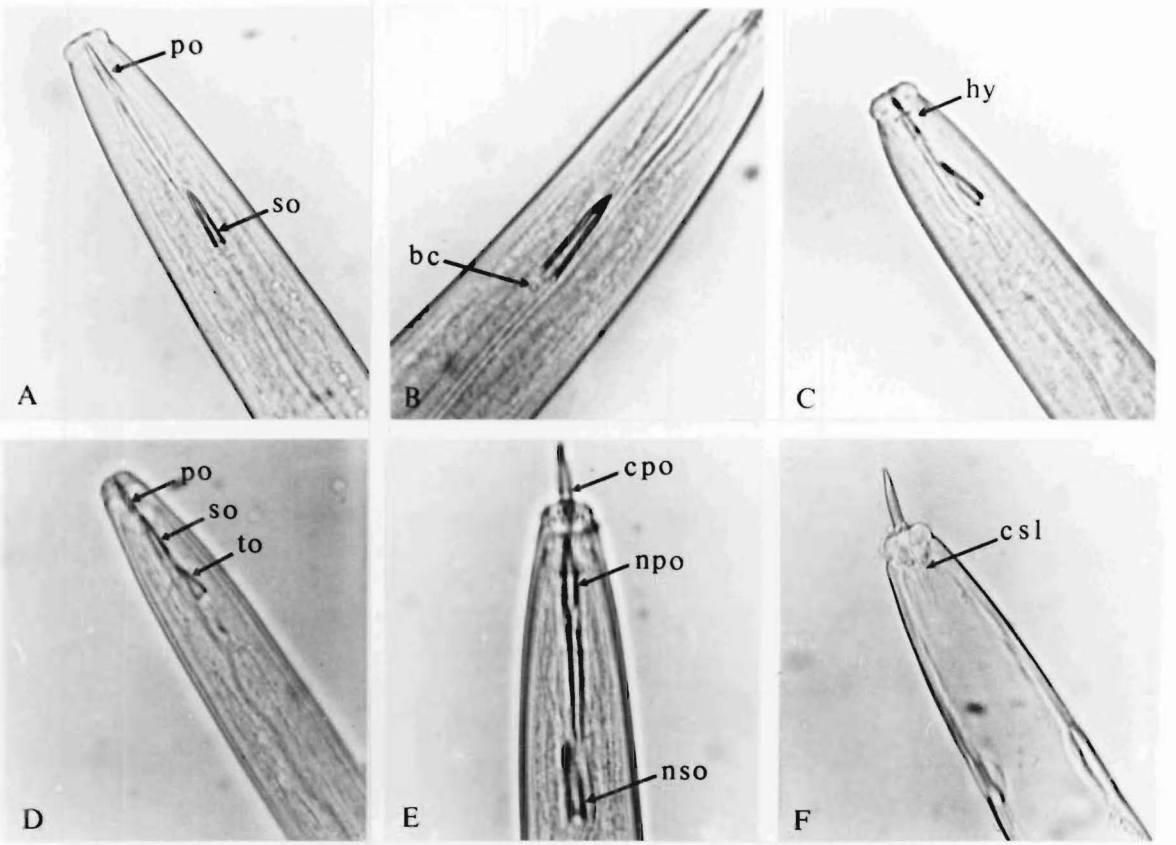
Third stage juveniles (n = 10): L=701-960μ (830); a = 21.9-24.4 (23.4); b = 2.8-3.9 (3.4); c = 41.3-57.5 (50.6); primary odontostyle = 13.5-15.0μ (14.2); secondary odontostyle = 16.0-19.0μ (18.4).

Fourth stage juveniles (n = 10): L = 805-1140μ (1025); a = 20.1-26.9 (24.8); b = 2.8-4.0 (55.1); c = 44.7-63.3 (55.1); primary odontostyle = 17.5-19.0μ (18.3); secondary odontostyle = 22.5-24.0μ (23.2).

Effect of temperature on egg hatching: None of the eggs incubated at 5°C (eight eggs), or 10°C (six eggs), hatched after six weeks. Development and hatching required 21-28 days at 15°C (six eggs); 10-12 days at 20°C (seven eggs), and 10-14 days at 25°C (six eggs).

Moulting: The approach of a moult can be predicted from the position of the secondary odontostyle relative to the primary odontostyle. Immediately before the moult the secondary odontostyle migrates from a position in the musculature of the oesophagus about two and one-half to three times its length behind the base of the primary spear shaft (Figure 35A and B), to lie just behind the primary spear (Figure 35C). Apart from occasional twitching of the body, movement ceases as the animal enters the moult. The contents of the intestine become coarsly granular, interspersed with globules of clear oil-like

FIGURE 35. Odontostyle replacement in moulting A. paraamylovorus.
A) Premoult position of the primary odontostyle (= po), and secondary odontostyle (=so). B) Location of stationary cell-like structure (= bc) at the base of the secondary odontostyle. C) Separation of new lip region evidenced by a hyaline area (= hy) around the primary odontostyle; secondary odontostyle has migrated to an anteriad position. D) Primary odontostyle forward of new lip region, secondary odontostyle located near its base, tertiary odontostyle (= to) formation evident. E) Separation of lip region completed, caste primary odontostyle (= cpo) of the previous stage is extruded; new primary odontostyle (= npo) in position, new secondary odontostyle (= nso) migrating posteriad. F) Exuviae (csl= caste spear shaft linings).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material. A hyaline area appears around the primary odontostyle (Figure 35C) before a faint outline demarcating the new head is seen, and the primary odontostyle of the previous stage is pushed forward (Figure 35D). About this time, the tip of the third odontostyle (new secondary odontostyle) becomes apparent close to the base of the new primary odontostyle. It slowly grows posteriad (Figure 35D), and is completed before the end of the moult. No definite spear forming cell was observed, but the tissue between the base of the sides of the developing spear shaft is more granular in appearance than the surrounding tissue. During the final stages of the moult the new secondary odontostyle migrates posteriorly a distance approximately equivalent to its length (Figure 35E). In its final position the base lies 2-3μ anterior to a prominent oval stationary cell-like structure which had occupied the same position relative to the previous secondary odontostyle (Figure 35B). Separation of the cuticle in the tail and anal regions follows separation of the cuticle around the lips. The cast spear, with its guiding ring is extruded about 12 hours before the moult is complete (Figure 35E). Longitudinal and rotatory motion of the juvenile within the cuticle begins, and just before the completion of the moult protrusion of the new primary odontostyle and pumping of the oesophageal glands occurs. The juvenile ruptures the cuticle behind the lips and down the side of the oesophageal region, emerging through the resultant pocket. The cast primary spear, the guiding apparatus and the old lining of the spear shaft are apparent in the exuviae (Figure 35F).

The pattern of moulting is the same for the second and third moults. In the first moult the secondary

odontostyle is located just behind the mouth spear and hence forward migration does not occur. In the fourth and final moult, a secondary odontostyle is not formed, and the cell associated with the final position of the new secondary odontostyle in previous moults is not apparent.

Post-moult defaecation: Nematodes raised on cultures of Haematococcus invariably contain dark red haematochrome in their intestine at the time of moulting (Figure 33A). On completion of the moult the material fills the posterior half of the intestine (Figure 33B). Subsequent post-moult activity is limited until the nematode expels its gut contents. This may occur between 30 minutes and seven hours after emergence from the moulted cuticle (six observations). Defaecation is usually preceded by a short feeding period during which one or two cells may be ingested (Figure 33C-E), but the nematode becomes sluggish again before the gut contents are expelled. Prior to expulsion, arching of the body is evident and movement of material within the intestine indicates that muscular contractions occur. The haematochrome is expelled through the anus to form a readily identifiable smear within the medium (Figure 33F). Feeding and migratory activity then resume.

Despite long periods of continuous observation, defaecation of solid material from animals was never observed during the active period between moults. Furthermore, solid material was never seen to pass into the pre-rectum during these observation periods.

The failure of attempts to follow the rate of digestion of food material using the red coloured haematochrome as a natural indicator add further evidence to the conclusion that non-digested solid material is retained in the intestine between moults. By transferring

animals from Haematococcus to cultures of Protosiphon, individuals with intestinal contents consisting of haematochrome in the posterior end and neatly demarcated chlorophyll in the anterior end were obtained. The red colouration at the posterior end of the intestine was retained several days later. Similarly, nematodes containing haematochrome transferred to sterile water agar and starved for up to two weeks retained the red colouration throughout. Activity of the animals was reduced, but no evidence of defaecation was observed.

Feeding prior to defaecation does not appear to be obligatory. One moulting animal was observed in which cuticle separation in the lip region was incomplete. Partial defaecation of the gut contents was indicated by the fact that the pre-rectum and the posterior part of the separated cuticle were filled with haematochrome.

DISCUSSION

The feeding process of A. paraamylovorus is similar to the pattern described by Lindford and Oliviera (1937) for dorylaims generally, and by Hollis (1957) for Eudorylaimus ettersbergensis.

In host range trials, populations of A. paraamylovorus were established on large spherical forms of algae, but not on small spherical species, or spindle or rod-shaped types with small diameter. As only cell contents are ingested (no entire algae cells were ever observed in the intestine) and the feeding process is stimulated by penetration, it appears that small cell size is the limiting factor. Hollis (1957), suggested that the diameter of the diagonal opening of the odontostyle tip may be related to the lower size limits of cells that

nematodes can attack successfuly. The internal diameter of the widest part of the diagonal opening of the odontostyle (x), and the external diameter of the shaft (y) for the five stages of A. paraamylovorus are as follows: First stage juveniles, x = 0.5μ , y = 1.0μ ; second stage juveniles, x = 0.7μ , y = 2.2μ ; third stage juveniles, x = 1.0μ , y = 2.7μ ; fourth stage juveniles, x = 1.2μ , y = 3.1μ ; adults, x = 2.0μ , y = 3.5μ . Thus, the odontostyle is almost one-half times the diameter of many alga cells and under moist conditions penetration is virtually impossible. Furthermore, the rounded odontostyle tip would not be conducive to penetration of small objects.

There was no evidence to indicate that suction between lips and the cell surface was necessary for penetration, as was suggested by Dickinson (1959). Indeed the odontostyle was extruded to such an extent during penetration that lip contact was not achieved until penetration was effected. Once the odontostyle is fully inserted, lip contact occurs, and the presence of suction forces during feeding are indicated by the retention of the collapsed host cell wall on the lips after the spear is withdrawn.

The retention of solid material in the intestine between moults has not previously been reported in nematodes. Defaecation in Ascaris lumbricoides Linnaeus, 1758, occurs at intervals of about three minutes under experimental conditions and almost completely empties the intestine (Crofton, 1966). Yeates (1969b) reported emptying of the pre-rectum of Diplenteron potohikus Yeates, 1969 every second, and Siddiqui and Taylor (1969) observed defaecation of minute droplets through the anus of Aphelenchoides bicaudatus. It is generally stated that the anterior

portion of the alimentary canal is secretory and the posterior part absorptive with the implication that nematodes digest food taken into the lumen and then absorb the products of digestion (Crofton, 1966). Large nucleated cells suggestive of a secretory function occur throughout the intestine of A. paraamylovorus. Further, the intestinal lumen appears folded throughout. Digestion and absorption in A. paraamylovorus may be a continuous process occuring over the length of the intestine during the inter-moult periods. Some modification of the ingested material is indicated by the change in the appearance of the contents of the posterior portion of the intestine and by the continued intake of food into an apparently filled gut. In such a system the pre-rectum probably functions in the release of liquid products of feeding and digestion, and in the control of the body cavity turgor pressure. The contents of the intestine undergo considerable change in appearance during the initial stages of the moult and this modification may necessitate its removal before digestive processes resume during the next juvenile stage.

The generation time of A. paraamylovorus is long compared with many free-living nematodes. Ferris (1968) reported similarly long generation times for Labronema ferox Thorne, 1939 (three to four months), and Labronema thornei Ferris, 1968 (five to six months). Observations on species of Eudorylaimus Andrassy, 1959, Nygolaimus Cobb, 1913, and Aporcelaimus made during the present study indicate that intervals of a similar order are required for the completion of a generation of these species. In all of the genera studied the duration of each stage was influenced greatly by the availability of food and the condition of the medium. When conditions

become unfavourable the nematodes assume a spiral position similar to that described by Hollis (1957) for E. ettersbergensis in drying agar. The tolerance of adverse condition in an inactive form may be an important survival mechanism in many dorylaimid species, and may prolong the generation time in the soil considerably.

Lifting of the outer egg shell membrane during hatching has been reported by Flegg (1968) for Xiphenema diversicaudatum (Micol., 1927) Thorne, 1939. Flegg (1968) considered that this may result from localised protein denaturation due to enzyme action. Immediate flexibility of the inner layer beneath the 'blister' in A. paraamylovorus, suggests that the outer membrane imposes the rigidity on the shell. The juvenile pushes into the blister and gradually the entire shell becomes resilient. Wilson (1958) suggested that larval movement before hatching helped to emulsify the inner lipoid layer of the shell, allowing osmotic intake of water and thereby generating pressure within the egg. The movement of liquid into the odontostyle of A. paraamylovorus during pumping of the oesophageal valve would increase body turgor and may be effective in increasing body size until the breaking tension of the egg membrane is reached.

Coomans and de Coninck (1963) studied the process of odontostyle formation in Xiphenema species. The pattern of formation of the new odontostyle close to the basal portion of the 'old' spear during moulting, with posteriad migrating after its completion was the same for Xiphenema and A. paraamylovorus. According to Thorne (1939), the odontostyle of members of the Dorylaimoidea originates in a special cell in one of the sub-median walls of the oesophagus. Coomans and de Coninck (1963) refer to an odontostyle forming cell,

probably with a secretory function, the nucleus of which migrates during the formation of the new odontostyle. No definite odontostyle forming cell was observed in live, of fixed specimens of A. paraamylovorus, but the granular appearance of tissue surrounding a forming odontostyle may be indicative of a secretory function. However, a prominent oval cell-like structure occurred in a position close to the base of the secondary odontostyle in the inter-moult stages. Migration of the structure did not occur during the moult. Newly formed odontostyles always migrated to the same position relative to the oval body that the secondary odontostyle of the previous moult held prior to moving anteriad. The structure was not observed in adults, nor was a secondary spear tip or mucro which is formed during the final moult of some dorylaims (Coomans and de Coninck, 1963), seen in adult A. paraamylovorus. It is suggested that the structure may be associated with the path taken by the migrating odontostyle in moulting juvenile stages. Further work involving the examination of sections through animals at various stages of the moult is envisaged to investigate this possibility.

The location of the secondary odontostyle in the region of the basal part of the primary odontostyle in first stage juvenile dorylaims has been noted by previous workers (Lordello, 1955; Coomans and de Coninck, 1963). Lordello (1955) suggested that first stage juveniles of Xiphenema krugi would be unable to feed because of the proximity of the secondary odontostyle to the lumen of the basal portion of the primary odontostyle. Coomans and de Coninck (1963) showed that the anterior part of the secondary odontostyle lies within a cavity in the ventral portion of the primary wall of the basal portion of the primary odontostyle of first stage juvenile

Xiphenema sp. Hence they concluded that these juveniles could ingest food. First stage juveniles of A. paraamylovorus were observed feeding on numerous occasions, thereby confirming the opinion of Coomans and de Coninck.

4.9 TYLENCHOLAIMUS SP.

INTRODUCTION

Tylencholaimus sp., was frequently isolated from soil from Broken River. The species was readily cultured on fungus hyphae growing in soil extract agar or cornmeal agar (see Chapter 3). Ferris (pers. comm., 1970) cultured Tylencholaimus teres Thorne, 1939 on fungus mycelium, but no detailed life-history studies have been reported.

METHOD

Host range: Rhizoctonia solani, Ulocladium atrum, Ascochyta sp., Fusarium oxysporum, Sclerotinia sclerotiorum, Stemphylium botryosum, Alternaria tenuis, Trichothecium roseum, and Zygorrhynchus moelleri, cultured on one-half strength cornmeal agar in 9.0cm Petri plates incubated at 20°C, were tested as suitable food for Tylencholaimus sp. Two plates for each fungus were inoculated with 10 Tylencholaimus, comprising a mixture of adults and pre-adults. A fungus was recorded as a favourable host if developing eggs and active juvenile nematodes were observed after six weeks incubation at 20°C.

Feeding and life-history: Observations on feeding and life-history were made on animals cultured on U. atrum on cornmeal agar or 1% water agar in glass ring observation chambers and 9.0cm diameter Petri plates.

RESULTS

MORPHOLOGY: Measurement of 10 females collected from soil: L = 530-630μ (577); a = 23.2-27.0 (24.3); b = 3.2-3.8 (3.4); c = 32.9-45.0 (38.7); V = 62-76% (67.4), G_1 = 26-49% (33.3); G_2 = 6-16% (10.6); odontostyle = 6-7μ .

Measurements of 10 females cultured on U. atrum on cornmeal agar: L = 690-850μ (749); a = 23.3-27.8 (25.1); b = 3.6-4.3 (4.0); c = 42.5-50.0 (45.2); V = 61.4-66.0% (64.1); G_1 = 20.8-29.4% (24.3); G_2 = 5.7-16.5% (11.0); odontostyle = 6.7μ .

A description of Tylencholaimus sp. was considered unsatisfactory until further work can be carried out for the following reasons:

i) Difficulties were encountered in the preparation of good quality specimens mounted in glycerine;

ii) Variations in gonad morphology were observed between specimens.

During dehydration, loosening of the cuticle and contraction occurred. In discussing the structure of the body wall of Dorylaimoidea, Siddiqui (1969) described the 'tylencholaimid' body wall as 'loose, often with fixation folds and radial elements. Its inner layer and subcuticle forming coarse annules'. The extent of the disfigurement of Tylencholaimus sp., varies between speciemens. By mounting large numbers of additional animals it is hoped that mounts of sufficient quality for descriptive purposes will be obtained.

Loof and Jairajpuri (1968) provided a key to females of Tylencholaimus which separated species into three groups; didelphic species, opisthodelphic species, and

prodelphic species. Specimens of the Broken River isolate of Tylencholaimus were initially considered to be didelphic. However, several specimens were isolated which possessed one developed ovary of the prodelphic form, with a rudimentary posterior gonad in evidence. The latter form is similar to T. airolensis Loof and Jairajpuri 1968, T. formosus Loof and Jairajpuri 1968, and T. stecki Steiner 1914, all of which Loof and Jairajpuri (1968) regarded as prodelphic species. Both forms of the Broken River Tylencholaimus species were observed in cultures. In old cultures and in animals approaching senility, the prodelphic form was dominant, but in cultures provided with an abundance of young hyphae as a food source, and in young adults, the didelphic form was dominant. It is considered that development of the posterior ovary is dependent on nutrition and age of the nematode. Hence, until confirmatory work is completed, the validity of gonad morphology as a major differentiating character between Tylencholaimus species must be regarded with reservation.

HOST RANGE: Tylencholaimus sp., fed and reproduced on all of the fungi tested.

PENETRATION AND FEEDING: Penetration of a hyphal cell wall is achieved with rapid thrusts of the odontostyle. Feeding begins immediately; hyphal cell contents are completely withdrawn following one to four pumps of the oesophageal valve. Dilation of the oesophageal valve plates coinciding with short backward movements of the oesophago-intestinal junction are involved in the pumping mechanism. Lateral movements of the head are sometimes necessary to facilitate removal of the odontostyle.

Feeding of Tylencholaimus sp., usually follows a pattern in which several cells in a row are ingested before the nematode moves to a new site (see Figure 36A-D).

Excretion of liquid material from the pre-rectum occurs at frequent intervals during feeding periods and during migratory activity. Filling of the pre-rectum is seen as a gradual enlargement of a hyaline area anterior to the anus (Figure 37A-B). Distension of the pre-rectum continues (Figures 37C-F) until it collapses (Figure 37G) and material is ejected through the anus. The defaecation cycle takes from 2 to 30 minutes; the frequency depending on the rate of feeding activity.

LIFE-HISTORY: Embryonic development: Eggs measure 58-66 x 35-37 μ at laying (15 observations). They are oval, with a rugose shell (Figure 38A). About 20 hours after laying the protoplast contracts at the mid-point, and divides to form two blastomeres (Figure 38B). Lateral division of the posterior cell occurs about six hours later, followed in one to two hours by division of the anterior blastomere to form a smaller 'dorsal' cell and a large 'ventral' cell (Figure 38C and D). Subsequent division of the 'ventral' blastomere (Figure 38D), is followed by division of the penultimate posterior blastomere (Figure 38E). About three and one-half to four days after laying, tissue differentiation is apparent as rapid division of the peripheral cells occurs in the anterior region (Figure 38F). During the following 20-30 hours further cell specialisation occurs, as the anterior region of the embryo becomes more hyaline and a tail fold appears (Figure 38 G and H). About five and one-half days after laying, the tadpole stage is apparent and movement of the

FIGURE 36. Feeding of Tylencholaimus sp., on Ulocladium atrum.
A-D) Sequential ingestion of cells of a hypha.

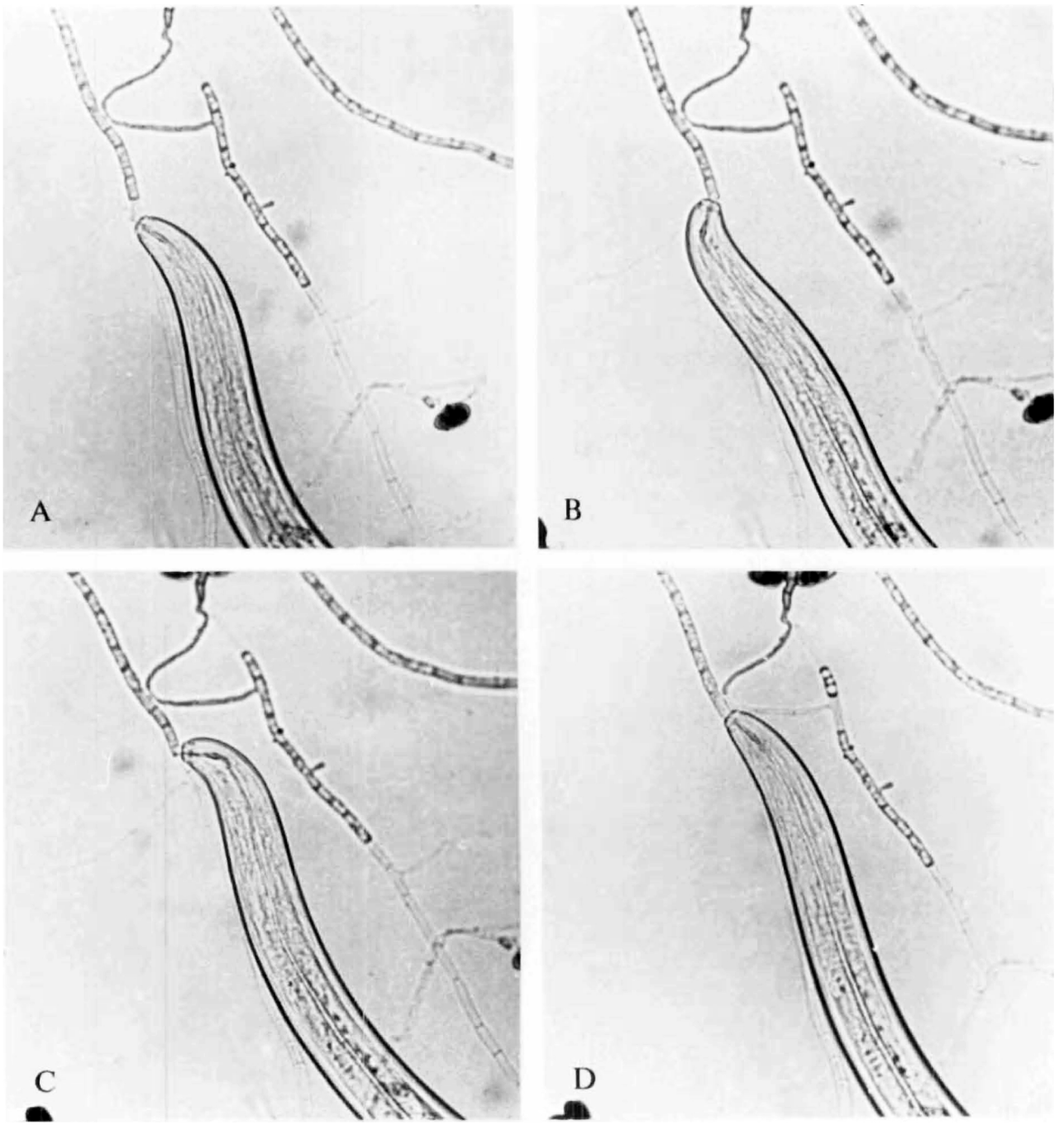

A
B
C
D

FIGURE 37. Defaecation of Tylencholaimus sp. A-F) Filling and distention of the pre-rectum (= prm). G) Collapse of the pre-rectum as defaecation occurs.

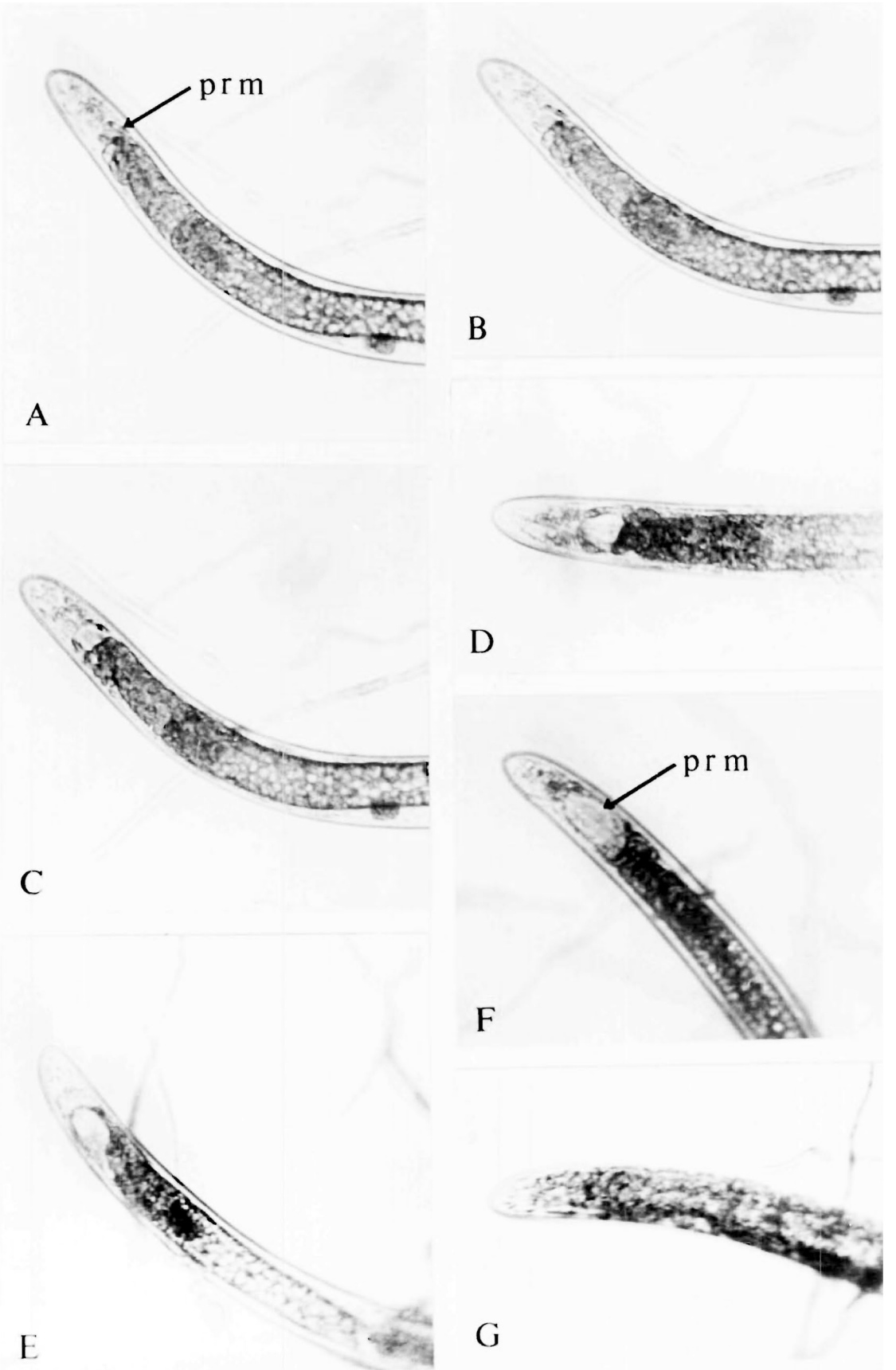
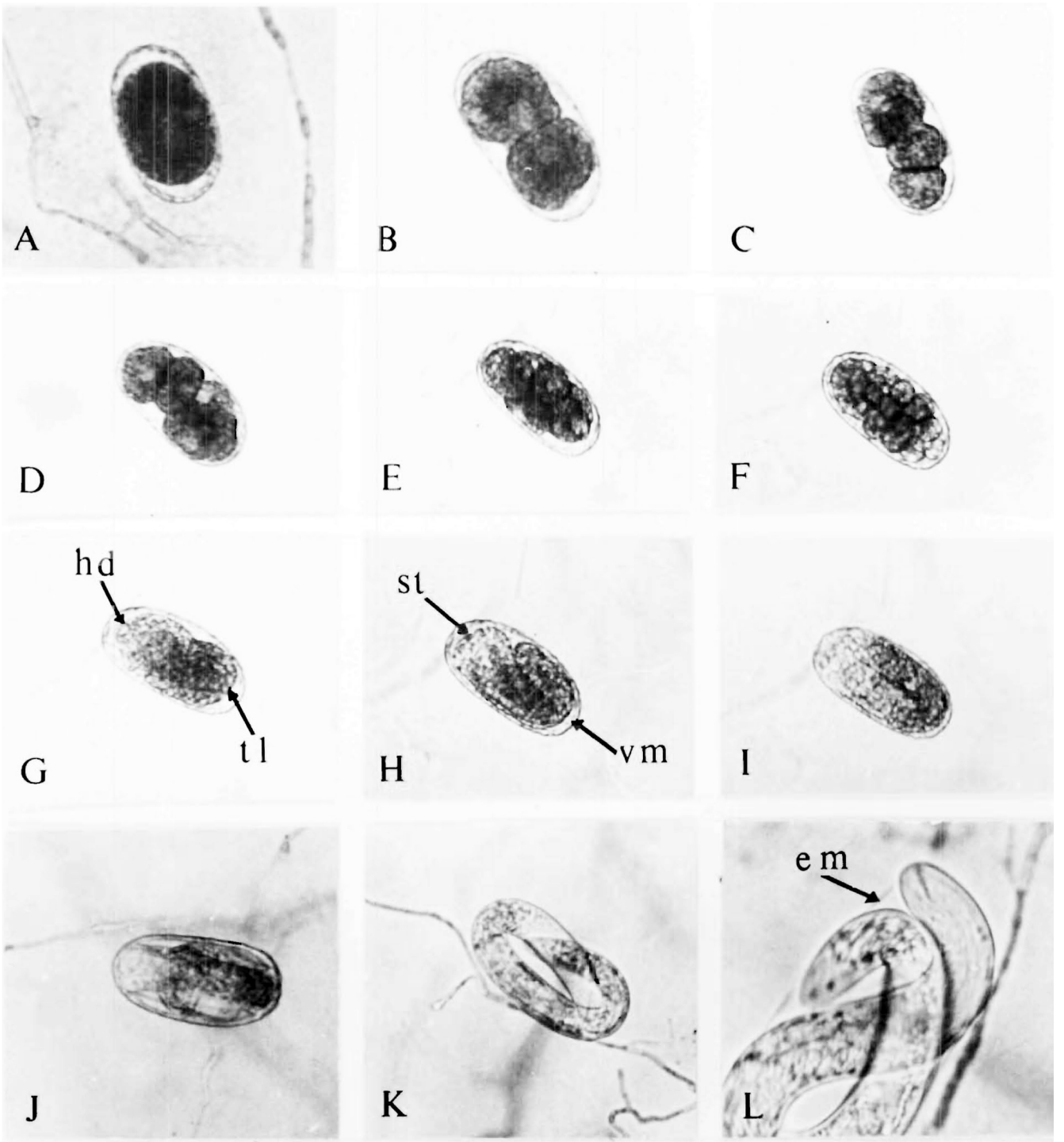

prm
A
B
C
D
prm
F
E
G

FIGURE 38. Embryonic development of Tylencholaimus sp. See text for explanation (hd = head end; tl = tail end; st = stomadeum; vm = vitelline membrane; em = egg membrane). Orientation of C) is reversed.


A
B
C
D
E
F
hd
tl
G
st
vm
H
I
J
K
em
L

embryo occurs. At this stage (Figure 38H) the anterior hyaline portion corresponding to the stomadeum and oesophageal regions are well formed and the embryo is still contained within the vitelline membrane. During the next 50-90 hours elongation of the embryo is evident (Figure 38I - J). Eight to nine days after laying, the juvenile is about three and one-quarter times the egg length long. The primary odontostyle is formed and about 2μ posterior to its base, the secondary odontostyle can be seen as a faint outline. Longitudinal and rotatory movement is continuous as the nematode flexes within the egg. About 20 hours before hatching, extension of the spear accompanied by pulsation of the oesophageal valve occurs. The intensity of body movement and pumping of the oesophageal valve increases over the next few hours, but no enlargement of the pre-rectum could be seen. During this stage the vitelline membrane becomes difficult to resolve and finally can not be seen. The presence of a folded transparent membrane within some eggs suggests that it may be ruptured by the abrasion caused during movement of the juvenile. About ten hours prior to hatching the walls of the egg appear thinner. Nematode activity is interspersed with frequent resting periods, but no moult was seen. During periods of activity pumping of the oesophageal gland and extrusion of the stylet are almost continuous and material was observed toward the spear orifice. About five hours before hatching, the egg shell becomes pliable and is gradually distended as the nematode flexes against the wall (Figure 38K). Immediately prior to hatching, the egg shell is evident as a thin pliable wall (Figure 38L) and it has increased in size by about 20%. Continued arching of the cephalic region and flicking of the tail stretch the wall until it ruptures about 9-12 days after laying.

Post-embryonic development: The duration of the first juvenile stage is short compared with later stages. It lasts 12-48 hours (10 observations) and on no occasion was feeding observed. Furthermore, juveniles which were hatched in the absence of fungi entered the first moult without feeding. Moulting first stage juveniles measure 234-265μ in length (10 observations). Second stage juveniles need to feed for development to continue. The second stage moult occurs 12-15 days after the first, at which time the juveniles measure 345-380μ (10 observations). The third moult occurs 13-15 days later (length of moulting third stage juveniles; 500-570μ), and the fourth, a further 18-24 days later (length of moulting fourth stage juveniles; 700-737μ).

On completion of the final moult, adult Tylencholaimus sp., may feed for several days before the first egg is laid. The interval appears to be related to the availability of food. On old soil extract agar cultures of U. atrum, up to 10 days elapsed before the first egg was laid. Whereas in fresh cornmeal agar cultures, laying has been observed after four days. Similarly, in fresh cultures females frequently contain an egg in each branch of the uterus, but near the end of the laying period and on old cultures, only the anterior uterine branch is functional. The number of eggs laid by the female has not been determined.

The generation time on U. atrum cultured on cornmeal agar at 20°C was seven to nine weeks.

EFFECT OF TEMPERATURE ON HATCHING: No eggs hatched after 40 days incubation at 5°C. At 10°C two of eight eggs hatched after 30 and 34 days respectively, but none of the remainder had hatched after 50 days. Eight of 10 eggs hatched after 15-20 days at 15°C; eight of ten

hatched after 9-12 days at 20°C, and seven from eight hatched after 7-10 days at 25°C.

DISCUSSION

The morphometrics of Tylencholaimus sp., varied between specimens raised in the laboratory, and specimens from the field. Pillai and Taylor (1967) reported similar variability in morphometrics of five mycophagous species reared on a range of fungi. No consistent correlation between host preference, host suitability and morphometrics was found in their experiments. Although genetic variation must account for much of the variability in natural populations, it is apparent that nutrition and environment are important factors which must be considered in taxonomic studies. The significance of nutrition effects are clear when the instability of gonad morphology as a diagnostic character for Tylencholaimus sp., is considered. Evans and Fisher (1970) reported changes in morphometrics of Ditylenchus myceliophagus Goodey, 1958 associated with changes in nutrition; the progressive change of many characters reflected their sensitivity to nutrition changes. They (Evans and Fisher, 1970) noted that the fastest growing organ in the adult is the gonad, and this was the character which showed the greatest percentage change during their experiments. The need for an experimental approach to taxonomy, as advocated by de Coninck (1962) is clearly evident.

Pitcher and McNamara (1970) showed that the presence of a suitable food source is the main factor promoting reproduction of Trichodorus viruliferus Hooper. Clark (pers. comm., 1970) has observed that a single ovary form of Diplenteron potohikus in old cultures with poor food supplies; on transferring these nematodes to fresh

cultures a second ovary develops. The sensitivity of gonad development to nutrition levels may be significant in regulating population increases of Tylencholaimus sp. If reproduction is restricted by low levels of nutrition, it follows that less pressure would be applied to existing nematodes in competition for food.

The mechanics of the feeding process of Tylencholaimus sp., are the same as those described for A. paraamylovorus. There was no evidence of extra-oral secretory activity, and parasitised cells collapsed after their cell contents were ingested during feeding. But the systematic pattern of feeding behaviour differed from that of other mycophagous species observed in the present study. By feeding on a number of neighbouring cells, and thus destroying large sections of hyphal strands, Tylencholaimus sp., may play a more significant role in the devastation of soil fungi. Destruction of hyphae in this manner may have serious effects on plant growth where mycorhizzal associations are necessary for plant development.

Defaecation of Tylencholaimus sp., follows the pattern described by Yeates (1969b)for Diplenteron potohikus (Yeates 1969), although the rate of emptying and refilling of the pre-rectum is considerably slower in Tylencholaimus sp. Yeates (1969b)was of the opinion that the pre-rectum acts as a control, permitting only the contained volume to escape at defaecation, thereby preventing indiscriminate or total escape of intestinal contents and allowing maximum utilisation of food. Mapes (1965) reported that defaecation of Panagrellus silusiae (de Man, 1913) T. Goodey, 1943 occurred at a critical body volume. In Tylencholaimus sp., the critical factor appears to be the volume of the pre-rectum.

The defaecation process differs in species not

possessing a pre-rectum (Siddiqui and Taylor, 1969; Yeates, 1969b). In Aphelenchoides bicaudatus muscular contractions in the posterior one-third of the body, resulted in defaecation of minute droplets through the anus (Siddiqui and Taylor, 1969). These workers were able to observe movement of ingested food in the intestinal lumen when A.bicaudatus was feeding on the green alga Stichococcus bacillaris and they noted a greater accumulation of ingested food in the anterior part of the lumen. Because Tylencholaimus sp., fed only on hyaline protoplasm of fungus hyphae, movement of material in the intestine could not be accurately followed. However, in A. paraamylovorus, which also posesses a pre-rectum, it was noted that the red and green coloured contents of Haematococcus cells accumulated in the posterior section of the intestine (see section 6.8). The presence of a pre-rectum may therefore promote more efficient utilisation of food as suggested by Yeates (1969b),by retaining material longer in the gut, and by allowing digestion to occur throughout the lumen. In this regard, it may be of significance that most of the larger long-lived free-living nematodes (e.g. dorylaimids) possess a pre-rectum.

Intensive activity of juveniles prior to hatching, accompanied by ingestion of liquid, has been noted for several species of nematodes in the present chapter. Ingestion of liquid by Tylencholaimus juveniles without defaecation would result in an increase in internal hydrostatic pressure. Harris and Crofton (1957) suggested that the cylindrical nematode body, equipped with longitudinal, but no circular muscles, must be turgid while retaining flexibility to provide the necessary force against which muscles work. Increase in body liquid causing an increase in turgor pressure, muscle tonus,

and possibly an increase in size, may assist Tylencholaimus juveniles during flexing and stretching of the egg shell prior to hatching.

No lifting of the outer membrane of the egg before hatching, was observed in Tylencholaimus eggs. In many nematodes pulsations of the oesophageal valves are often accompanied by an outward flow of secretions from oesophageal glands which may cause chemical changes to the egg membranes (Taylor, 1962). Although no secretion of material from the stylet of pre-hatch Tylencholaimus juveniles was seen, the egg shell became thinner and flexible as nematode movement and pulsation on the oesophageal valve occurred. This may be indicative of an undetected chemical action.

Tylencholaimus sp., undergoes four post-hatch moults before reaching adulthood. The first moult, which occurs within a few hours of hatching, approximates the pre-hatch moult characteristic of Tylenchida. This fact, together with the 'tylenchoid' appearance of the odontostyle and the mycophagous habit of the species, suggests a 'bridging' relationship between the Orders Tylenchida and Dorylaimida.

4.10 ACROBELOIDES SP.

INTRODUCTION

Many workers have raised populations of soil nematodes on culture media supporting colonies of bacteria (Nigon, 1949; Nicholas, 1959, 1962; Dougherty, 1960; Chuang, 1962; Cryan, Hansen, Sayre and Yarwood, 1963; Thomas. 1965; Rothstein and Clark, 1966; Pillai and Taylor, 1968a, 1968b; Sohlenius, 1968a, 1968b, 1969; Yeates, 1970). Some species of nematodes from Broken

River were shown to be bacterial feeding forms (see Chapter 3). One of the most abundant of these, Acrobeloides species was considered in further experiments to investigate its generation time, the effect of temperature on egg hatching time, and to determine the suitability of a number of bacteria species as food for the nematodes.

METHOD

Stock populations of Acrobeloides sp., were maintained on a mixed flora of bacteria from Broken River soil, cultured on cornmeal agar.

Generation time: Five gravid females were placed on the surface of 1.5% soil extract agar in 15, 4.5cm diameter Petri plates. The surface of the agar was inoculated with a lawn of mixed bacteria from the stock cultures 48 hours prior to the introduction of nematodes. After 12 hours the adults were recovered from the plates and all but five eggs removed. The cultures were incubated at 20°C and nematode development observed daily until maturity of a female in each plate was indicated by the presence of eggs. Maturation time of the first female only was recorded.

Effect of temperature on hatching time: Several 4-5cm diameter Petri plates containing soil extract agar, a mixed bacterial flora, and five eggs of Acrobeloides sp., were prepared as above. Three plates were incubated at each of the following temperatures: 5, 10, 15, 20 and 25°C. Regular observations were made to determine the time required for egg hatching.

Host range: Cultures of test bacteria were obtained from the Department of Microbiology and Genetics, Massey University, and from the Botany Department, University

of Canterbury. Nine centimetre diameter Petri plates containing soil extract agar and one-half strength nutrient agar, were inoculated with bacteria by streaking over one-half of the area of the plate. The cultures were incubated for 48 hours at 20°C before nematodes were introduced. Nematodes were rinsed from the surface of stock cultures, sterilised, and 20 animals placed on each of four plates (two plates for each medium) per species of bacterium. After incubation at 20°C in continuous dark for 20 days, the plates were examined and the food organisms assigned an 'index of suitability' based on a visual assessment of the abundance of nematodes in the cultures.

RESULTS

Generation time: Acrobeloides sp., requires 13-16 days to complete one generation at 20°C. Embryonic development required 3-5 days; post-embryonic development, 9-12 days.

Effect of temperature on egg hatching time: Embryonic development was completed after 4-5 days at 25°C (100% hatch); 3-5 days at 20°C (93% hatch); 9-11 days at 15°C (75% hatch), and 19-25 days at 10°C (47% hatch). Eggs incubated at 5°C failed to mature.

Host range: Acrobeloides sp., established populations on all but B. cereus var. mycoides of the food organisms tested. The 'suitability' of bacteria varied between species, and between culture media (Table 19).

TABLE 19: Effect of food organisms and culture media on population development of *Acrobeloides* sp.

Food organism	Population development on	
	½ strength nutrient agar	Soil extract agar
Escherichia coli	***	**
Proteus vulgaris	***	**
Pseudomonas fluorescens	**	**
Bacillus megaterium	**	**
Bacillus cereus	*	**
B. cereus var *mycoides*	-	-
Chromobacterium sp.	-	*
Flavobacterium sp.	*	*
Erwinia sp.	*	*
Aerobacter aerogenes	***	**
Corynebacterium sp.	**	*
Sarcina sp.	*	*
Serratia marscescens	*	*

*** dense populations developed

** moderate populations developed

* reproduction occurred, but populations comparitively small

- no populations developed

DISCUSSION

The generation time of *Acrobeloides* sp., is slightly longer than the interval of 9-10 days at 25°C reported by Nicholas (1962) for *Acrobeloides buetschlii* (de Man, 1884) Steiner and Buhrer, 1983. Eggs hatched at 10°C but the percentage hatching was reduced at this temperature. Thus population replacement is unlikely to be significant at temperatures below 10°C.

Acrobeloides sp., reproduces on a wide range of food organisms under culture conditions. The 'suitability' index is subject to the culture environment. Sohlenius (1968a) observed similar variations in culture experiments with *Diplogaster nudicapitatus* Steiner 1942, and *Rhabditis maupasi* Seurat 1919. Because of the media influence, comparison of suitability between food species is of doubtful value, but relative non-specificity of feeding habits is indicated. Sohlenius (1968a) found that some bacteria were unsuitable as food for *D. nudicapitatus* and *R. maupasi*, which he suggested may be related to structural or chemical factors. *Acrobeloides* sp., did not reproduce on *B. cereus* var. *mycoides*, but established populations on the closely related form *B. cereus*. The 'mould-like' aggregate formed by *B. cereus* var. *mycoides* on agar suggests that the firm structure of the colonies limits the availability of potential food cells.

4.11 NEMATODE-TRAPPING FUNGI

INTRODUCTION

Predacious fungi have been reported from many areas of the U.S.A., England and U.S.S.R., and from France, North Africa, Denmark, Germany, Austria, Hungary, Palestine

(Israel) and the Pacific Islands (Supronov, 1966). They are found with regularity in leaf-mould, rotting wood, soil, dung and decaying plant material of many kinds (Duddington, 1955).

Several workers have investigated the occurrence of predacious fungi in soil (Drechsler, 1937; Lindford, 1937c;Duddington, 1954; Shepherd,1956; Mankau and Clark, 1959; Tolmstoff, 1959; Mackenzie, 1960; Mankau, 1962; Morton, 1963; Pramer, 1964; Estey and Olthof, 1965; Supronov, 1966), Fowler (1970) reported the results of an extensive survey of predacious fungi from 62 sampling stations located throughout New Zealand. Eight Hyphomycete species, two Zoopagales, and five endozoic parasites were identified from 609 records from 56% of the 700 samples taken. In contrast to Fowler's survey, the present investigation was designed as a more intensive survey of the incidence of nematode-trapping fungi in the study area. In the following Chapter, the possible relationship between the presence on nematode-trapping fungi and numbers of nematodes is considered.

Six species of predacious fungi were identified from Broken River soil, including an undescribed species of Harposporium Lohde.

Several opinions have been expressed regarding the manner of infection of Harposporium species. Kostka (1927) considered that the sharp apices of conidia penetrated the nematode cuticle as a result of pressure applied during movement. Kühnelt (1961), cited H. anguillulae Lohde, 1874 (Karling, 1938), as an example of those fungi with pointed spores that "bore into the skin of nematodes". On the other hand, Drechsler (1941) suggested that the spores of H. oxycoracum Drechsler 1941 attached to the body of the prospective host by means of a sticky

fluid on the conidial tip, but he did concede that the cuspidate conidial apiculum of the fungus might be involved in hooking the spore to, or into the nematode. Duddington (1955) expressed the view that spores of H. anguillulae adhere to the nematode cuticle, and later (Duddington, 1957; 1962) suggested this mode of attachment for most other species of Harposporium. Further, conidia of H. subuliforme Drechsler, 1950 were described as attaching to the nematode integument by means of an adhesive spur produced on the distal end of the spore (Drechsler, 1950). But both Duddington (1957) and Drechsler (1946, 1954) note that infection by H. bysmatosporium Drechsler 1946 is achieved when conidia become lodged in the stoma of the host eelworm during feeding. Similarly, infection by the oral route was observed for H. baculiforme Drechsler 1959, and H. sicoydes Drechsler, 1959, although in some infected animals the avenue of attack was considered to be conjectural (Drechsler, 1959). Drechsler (1963) suggested the same mode of nematode infection for H. dicorymbum Drechsler, 1963. Aschner and Kohn (1958) showed that conidia of H. anguillulae entered an unidentified Rhabditis sp., through the stoma and became lodged in the upper regions of the digestive tract. On no occasion did they record evidence to support the view that infection resulted from adherance of spores to the nematode cuticle. Boosalis and Mankau (1965) likewise noted that isolates of H. anguillulae obtained from Californian soils must be injested by nematodes to become infective. This is consistent with the observations of Dixon (1952) who did not find eelworm with conidia of H. lilliputanum Dixon, 1952 adhering to the cuticle even in the early stages of an attack. Shepherd (1955) also noted that no living nematodes were found with conidia of H. crassum

Shepherd, 1955 attached to them. More recently, Barron (1970) showed that infection of nematodes by H. helicoides Drechsler, 1941, is achieved after feeding eelworms ingest the conidia. A prominent drop of mucus was present at the basal end of the spore, but no evidence of an adhesive function was seen (Barron, 1970).

In view of the varied mechanisms of nematode infection which have been suggested for some Harposporium species and the apparent confusion concerning others, particular attention was given to this aspect of the disease cycle in the present paper.

MATERIALS AND METHODS

Two series of samples were examined; the first series consisting of 10 samples was taken in May 1968 over a range of habitats, and the second of 20, obtained in October of the same year, was confined to soil from beneath fescue tussock plants.

Samples were taken with a soil corer of $5cm^2$ cross section to a depth of 7.5cm. From each core approximately 1g of soil was seeded to a petri-dish containing 15ml of Difco cornmeal agar. The petri-dishes were incubated at room temperature and examined weekly for 14 weeks.

For identification purposes material from nematode infested cultures was examined in temporary water mounts. Fifty conidia were measured for each of the fungi isolated.

Nematodes infected by a Harposporium sp., were studied in water mounts, and in lactophenol acid fuschin. The early stages of infection were observed in live animals and animals fixed in 5% formalin containing 0.04% acid fuschin.

RESULTS

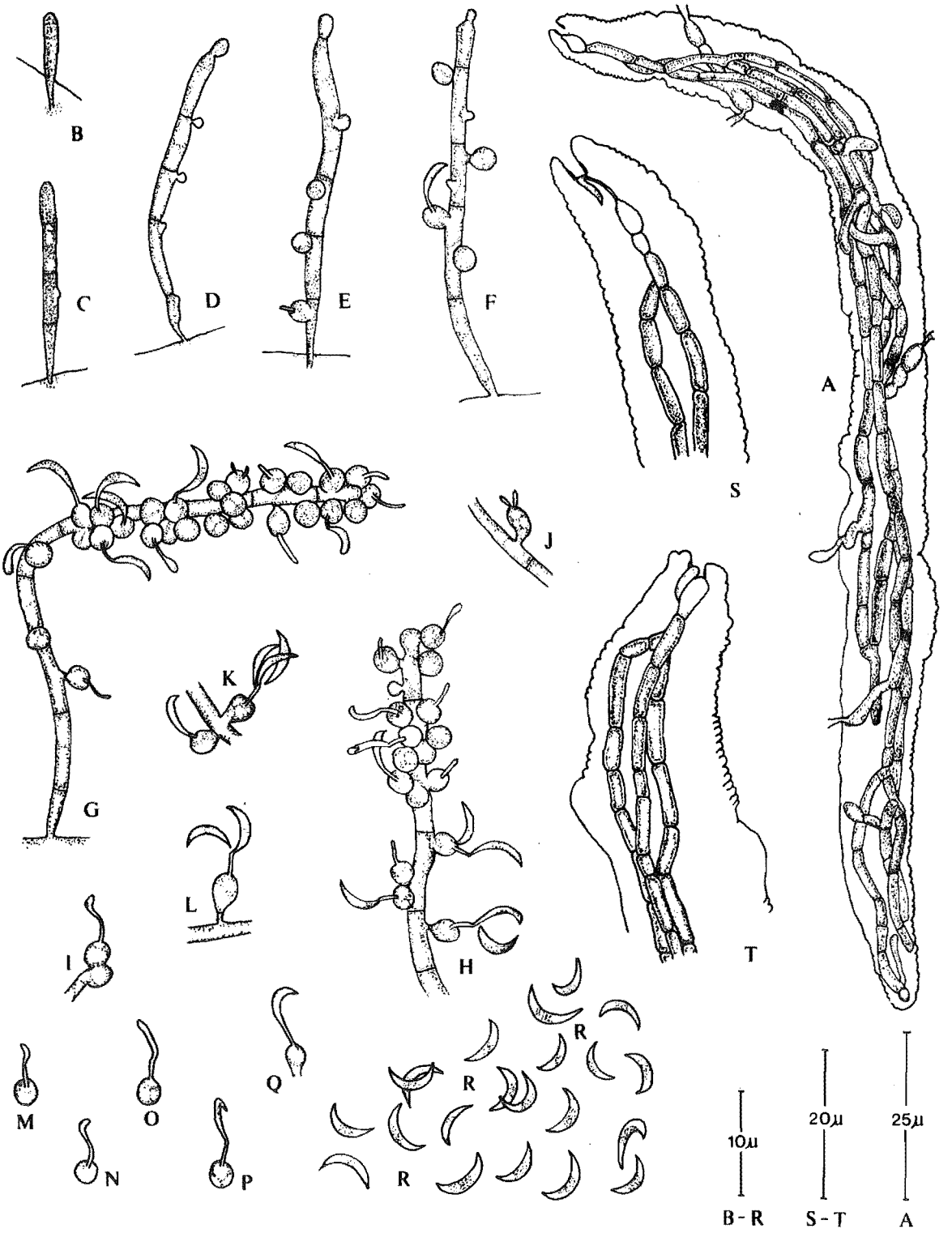
Six species of nematode-trapping fungi were recorded in a total of 14 isolations made from 33% of the plated samples (Table 20). Four of the species isolated, Acrastolagmus obovatus Drechsler 1941, Monacrosporium eudermatum Drechsler 1950 (Cooke and Dickinson, 1965), Arthrobotrys oligospora Fresenius (1852; Drechsler, 1937), and Monacrosporium cionopagum Drechsler 1950 (Subramanium, 1963), were recorded by Fowler (1970) from New Zealand soils.

Harposporium sp. nov. The endozoic mycelium is colourless, branched, 1.5-4.0μ wide, septate, mostly 5.0-10.0μ between septa, and completely permeates the eelworm cadaver (Figure 39A). Conidiophorous hyphae are formed externally, about 1.0μ wide at the point of exit, widening to 2.0-3.0μ , septate, mostly 5.0-15.0μ between septa, usually simple, 40.0-250.0μ in length, ramifying through the surrounding media. They are initiated as a unicelled outgrowth (Figure 39B) before septa are delimited and primordial conidiiferous cells or phialides appear as lateral buds along their length (Figure 39C-E). Phialides are usually produced directly, but sometimes on short stalks (Figure 39F-L) singly on young conidiophorous hyphae, in clusters on older hyphae (Figure 39F-H). They are spherical to subspherical in shape, 2.5-4.5μ in diameter, bearing a terminal sterigma, rarely two, 1.0-3.0μ long, about 0.5μ wide on which up to six arcuate conidia are produced (Figure 39F-L). Phialides on some occas ions proliferate through the apex to produce a second conidiiferous cell (Figure 39I). Conidia were produced by elongation and swelling of the sterigma before an obvious septum defined the spore extremeties (Figure 39M-Q). Mature conidia are hyaline, single celled, distally pointed, finely truncated at the base, 4.0-6.1μ (mean

TABLE 20: Incidence of predacious fungi from Broken River.

Month of sampling	No. of samples	No. of samples with predacious fungi	Species isolated	No. of isolations
May	10	3	Arthrobotrys oligospora	1
			Acrastolagmus obovatus	1
			Monacrosporium cionopagum	1
October	20	7	Arthrobotrys oligospora	2
			Acrastolagmus obovatus	2
			Monacrosporium cionopagum	3
			Monacrosporium eudermatum	2
			Monacrosporium parvicollis	1
			Harposporium tarum	1

FIGURE 39. Harposporium tarum n. sp.
A) Infected nematode cadaver permeated with trophic hyphae. B-F) Development of conidiophorous hyphae and phialides. G-L) Variations in the production and form of phialides and sterigma. M-Q) Production of conidia. R) Conidia. S-T) Penetration and infection arising from the buccal cavity.


B
C
D
E
F
A
S
G
J
K
H
L
T
I
M
O
Q
R
N
P
R
R
10µ
B-R
20µ
S-T
25µ
A

4.9μ) long and 0.8-1.2μ wide, slightly convoluted, describing up to one quarter of a turn (Figure 39R).

The only other species of Harposporium with arcuate conidia of such small dimensions is H. lilliputanum, but the conidia of H. lilliputanum are larger (Dixon, 1952) and are not usually pointed (Cooke and Godfrey, 1964). It is therefore proposed to erect a new species, H. tarum, the specific epithet from the Maori, tara meaning spike, referring to the spike-like terminal apices of the conidia.

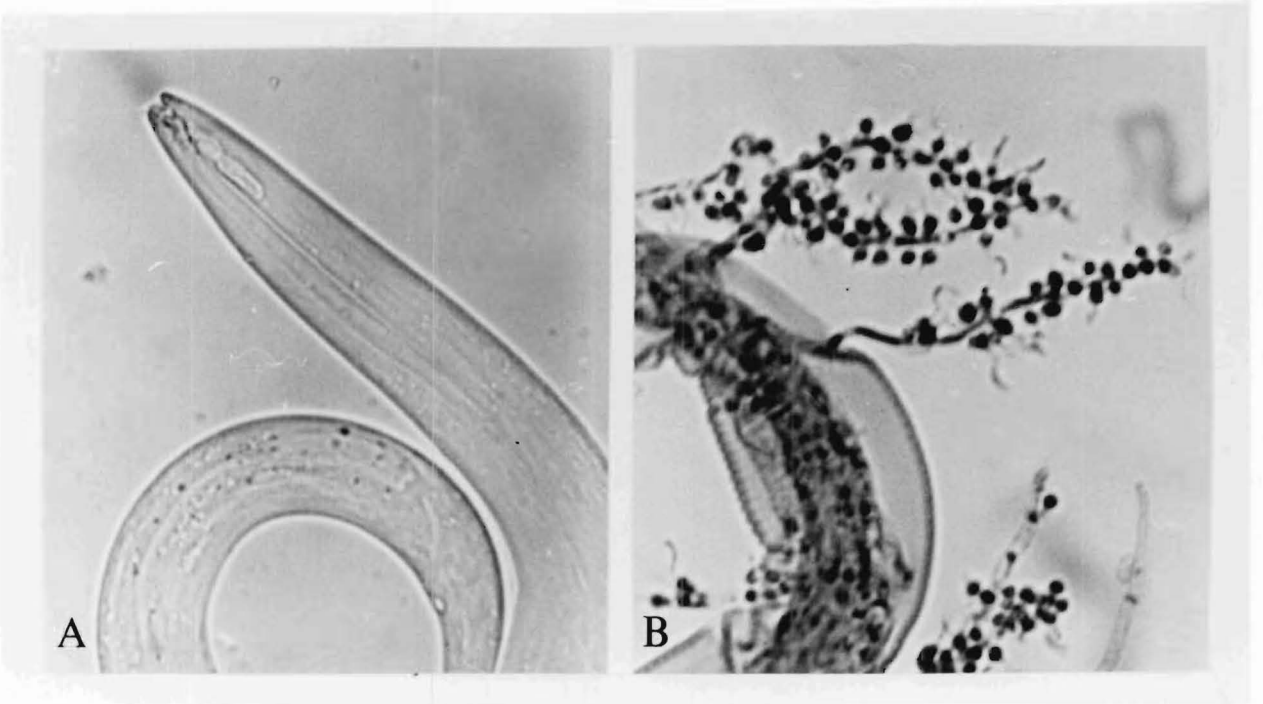
Habitat: From soil beneath Festuca novae-zelandiae.

Type locality: From soil around roots of Festuca novae-zelandiae at Broken River (map reference N.Z.M.S. 1, Sheet S66); altitude 720 m.s.l.

Conidia are ingested during feeding of Acrobeloides sp., Eucephalobus sp., and Rhabditis sp., The spike-like apices of the spores impede movement and cause them to lodge in the buccal cavity. The dentate dorsal metarhabdion appears to provide an obstruction which facilitates the lodgement of conidia; infections of these two species did not originate from below this point. Similarly, infections of the Rhabditis sp., originate from the stoma, usually near the glottoid apparatus, but on one occasion a conidium had penetrated to the upper regions of the oesophagus. A short germ tube up to 5.0μ long is produced which enlarges to form a trophic hypha within the oesophageal muscle tissue (Figures 39S and T; 40A). At this stage nematode movement becomes sluggish and stops. The assimilative hyphae may branch with the resultant strands growing in the same direction through the muscle tissue toward the oesophageal bulb. Conidiophorous hyphae are rarely formed until the trophic hyphae permeate the length of the cadaver. Infected cadavers

FIGURE 40. Infection of *Eucephalobus* sp., by *H. Tarum* and production of conidia on an infected cadaver.

A) Infection of *Eucephalobus* sp., arising from the buccal cavity. B) Production of conidia and 'grape-like' aggregations of conidiophorous hyphae in the medium surrounding an infected cadaver.


A
B

are readily identified by the presence of 'grape-like' aggregations of phialides on the conidiophorus hyphae in the surrounding media (Figures 40B and 41A). Chlamydospores were never observed, even in partially decomposed cadavers long after sporulation had stopped. In many infected eelworms, the assimilative hyphae appear to become more coarsly granulated as growth of the fungus slows, but there is no obvious distention of individual hyphal cells.

Acrastolagmus obovatus Drechsler 1941. Nematode cadavers were observed in several soil cultures with fine conidiophorous hyphae bearing phialides and conidia, radiating from them (Figure 41B).

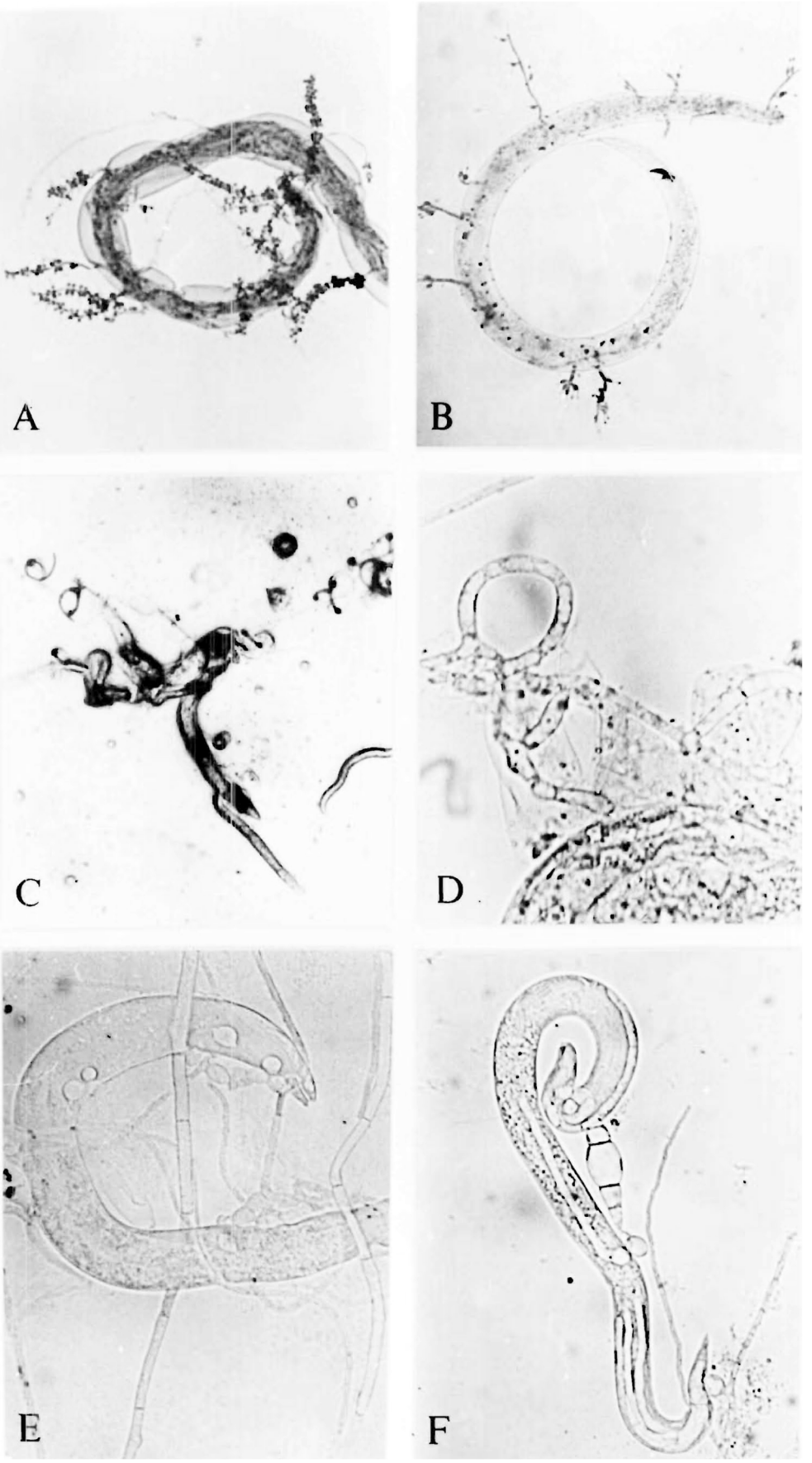
Conidia were ellipsoidal, 1.9-3.2 μ (mean 2.9 μ) by 1.8-2.5 μ (mean 2.1 μ) produced on flask shaped phialides 7.0-9.0 μ long, characteristic of those described by Drechsler (1941) for A. obovatus. Conidiophorous hyphae 1.5-2.4 μ wide, with 8.0-18.0 μ between septa, extended up to 800 μ into the surrounding media.

The fungus parasitized nematodes of the genera Acrobeloides, Chiloplacus, Eucephalobus, Wilsonema, Plectus, Aphelenchoides, Seinura and Tylenchus. Arthrobotrys oligospora Fresenius '1852; Drechsler, 1937).

A fungus was observed capturing nematodes in networks of modified hyphae produced on the surface of the medium and within the medium (Figure 41C). In some instances an entire nematode population was destroyed.

Sporulation was prolific, conidia being produced in whorls along aerial conidiophores. Conidia were typically obovoid, two celled, 9.0-29.0 μ (mean 25.2 μ) by 9.0-17.0 μ (mean 14.5 μ), conforming to the description of A. oligospora by Drechsler (1937). Adhesive hyphae extensive, consisted of a succession of cells 10.0-30.0 μ long by 3.5-6.0 μ wide, often branched

FIGURE 41. Nematode -trapping fungi isolated from Broken River.
A) Nematode cadaver infected with Harposporium tarum. B) Seinura demani infected with Acrostolagmus obovatus. C) Nematodes ensnared on the surface of a culture of Arthrobotrys oligospora. D) Adhesive network of Monacrosporium eudermatum. E) Nematode ensnared by branches of adhesive cells of Monacrosporium cionopagum. F) Nematode infected by Monacrosporium parvicollis after adhesion to knob-like cells produced on vegetative hyphae and conidia.


A
B
C
D
E
F

and frequently anastomosing with adjacent cells to form three dimenional networks of irregular loops.

Monacrosporium eudermatum Drechsler 1950 (Cooke and Dickinson, 1965).

Nematodes were captured through adhesion and entanglement in two dimensional networks of modified hyphae (Figure 41D). No discrimination between prey species was evident.

Conidia were borne singly on aerial conidiophores. They were broadly spindle shaped, distally rounded, tapering at the base, 2-4 septate, mostly three, measuring 35.0-51.0μ (mean 44.8μ) by 16.0-27.0μ (mean 22.6μ), which agrees with the description given by Drechsler (1950).

Monacrosporium cionopagum Drechsler 1950 (Subramanium, 1963).

One to two months after seeding soil samples to cornmeal agar, nematodes were observed trapped by adhesive branches of the sporulating fungus (Figure 41E).

Conidia were spindle shaped, rounded at the distal end, narrowing at the base, mostly 4 septate (range 2-5), 37.0-65.0μ (mean 56.0μ) by 15.0-22.0μ (mean 19.4μ), produced singly on aerial conidiophores. Adhesive branches consisted of one to many (usually 1-5) columnar cells, 4.0-8.0μ wide, constricted at the septa, sometimes anastomising with branches of the same of adjacent hyphae to form irregular networks of up to 20 cells.

Conidial measurements and hyphal characteristics are in accord with the description given by Drechsler (1950).

Monacrosporium parvicollis Drecshler 1962 (Cooke and Dickinson, 1965).

Capture of nematodes was effected by adhesion to knob-like cells (Figure 41F).

Conidia were fusiform to spindle shaped, 26.0-55.0μ (mean 43.1μ) by 7.5 - 15.0μ (mean 12.0μ), 2-5 septate, mostly 4, rounded at the distal end, proximally truncate and produced on aerial conidiophores. The extreme conidial measurements exceed the range recorded by Drechsler (1962) for M. parvicollis but fall within the limits recorded by Cooke (1967).

At frequent intervals along the vegetative hyphae, spherical to ovate cells 8.8-13.0μ long and 6.0-10.0μ wide were produced, usually on short stalks 2.0-4.0μ by 0.5-8.0μ . Conidia frequently germinated to produce knob-like cells, mostly from the apical or basal cell and these were effective in nematode capture (Plate 1F). On older hyphae, the knob cells occasionally proliferated to form short chains of adhesive cells which sometimes anastomosed with the same or adjacent hyphae or with a second proliferation to form an arch or semi-loop structure.

Like Cooke's (1967) culture, the New Zealand isolate sporulated well in pure culture on cornmeal agar.

DISCUSSION

Nematode-trapping hyphomycetes were abundant in tussock grassland soils from the vicinity of Broken River, as evidenced by the frequency of their isolation. It would be foolish to suggest that the six species identified comprise the total predacious fungus flora of the area. For example, in nematode samples from Baermann Funnel extractions a specimen of Aphelenchus avenae was observed ensnared in a trapping device defined by Cooke and Godfrey (1964), as a constricting ring, but on no

occasion was such a fungus identified in the cultures from soil samples. But, it does appear that A. obovatus, A. oligospora, M. eudermatum, and M. cionopagum are among the most commonly occurring species.

Characteristic globose phialides and arcuate conidia are produced by H. tarum. The development of conidiophorous hyphae, conidiiferous cells, and conidia closely follows the pattern described by Karling (1938) for H. anguillulae.

Conidia of H. tarum effect entry into nematodes through the stoma during feeding. The beak-like extremeties of the spores become lodged in the walls of the buccal cavity and a germ tube gives rise to trophic hyphae from this point. Ingestion of fungus conidia by saprozoic nematodes has been shown to occur for fungi other than nematode-trapping forms. Jensen (1967) reported that Pristionchus (Diplogaster)lheritieri (Maupas) Paramonov, Panagrellus redivivus (Linn.) Goodey, Panagrolaimus subelongatus (Cobb) Thorne, and Rhabditis spp., ingested spores of various plant pathogenic fungi. The predacious habit of H. tarum depends on the non-selective feeding of such nematodes; the crescentic shaped conidia with their pointed tips act in the manner of a fish hook as suggested by Aschner and Kohn (1958) for H. anguillulae. All of the nematode species infected were bacteriophagous, stylet bearing nematodes were never attacked, and nematodes were never observed with conidia attached to the cuticle. Barron (1970) observed spores of H. helicoides germinating in both the upper and lower gut regions of parasitised nematodes, 'in no case were incipient infections found originating from a spore lodged in the muscle tissue of the oesophagus'. Penetration and infection of nematodes by H. tarum usually occurs in the stoma. The consistent origin of infection hyphae from a

position close to the glottoid apparatus suggests that the structure of the posterior part of the buccal cavity may partially obstruct the movement of conidia. The glottoid apparatus is the first major constriction of the alimentary canal the chances of penetration from this position are increased.

Ingestion of spores is necessary for nematode infection by H. anguillulae (Aschner and Kohn, 1958), H. helicoides (Barron, 1970), and H. tarum. From a survey of the literature it can be seen that in reports of nematode infections by Harposporium spp., for which the host is recorded, non-stylet-bearing nematodes are implicated (Drechsler, 1941, 1954, 1959, 1968; Aschner and Kohn, 1958; Barron, 1970). Furthermore, the evidence indicates that H. lilliputanum (Dixon 1952), H. crassum (Shepherd, 1955), H. sicoydes and H. dicorymbum (Drechsler, 1959) infect nematodes through the oral route. Apart from H. subuliforme in which conidia adhere to the eelworm cuticle (Drechsler, 1950) there appears to be no positive record of cuticular penetration by Harposporium spp. H. subuliforme differs from other Harposporium spp., in two additional features; conidiophorous cells depart from the sub-spherical type characteristic of Harposporium spp., and conidia germinate soon after they are cut off to produce a process by which they adhere to nematodes. Drechsler (1950), recognised the peculiarities of his fungus, but considered that the characteristics of the newly formed conidia justified its inclusion in the genus Harposporium. It would appear from the evidence that the placement of H. subuliforme may require further consideration.

4.12 GENERAL DISCUSSION

The ecological diversity of nematodes is well illustrated by the biological variation between species which has been shown in the present chapter. Variation in feeding habits, generation time, post-embryonic development and general behaviour may be marked even for species of closely related genera (e.g. A. bicaudatus and S. demani; A. neozelandicus and T. rikus).

All of the nematode species studied exhibited wide host ranges within a particular food type. Furthermore, the approximate lower cardinal temperature for embryonic development occurs at, or about, 10°C for most species (Table 21). Dao (1970) notes that the effects of temperature on survival and thrift of nematodes differs markedly between species and between populations of species, but that specific populations appear to be temperature adapted to their own climate. Nematodes at Broken River appear to be similarly adapted. Variation between species no doubt occurs, but some degree of adaptation to a common environment is indicated. However, the existence of 'biological species' shown by Dao (1970) and suggested for A. bicaudatus (section 4.5.), illustrates the need for laboratory studies of local populations for the interpretation of field data in ecological and plant pathological considerations. In addition, it has been shown that biological studies can play an important role in taxonomic considerations. Variability of important diagnostic characters was shown for some species (e.g. A. bicaudatus and Tylencholaimus sp.). Under laboratory conditions large numbers of animals may be cultured in a controlled environment. Observations on these animals, together with specimens from the field, enables a more realistic evaluation of diagnostic characters.

The ability of nematodes from Broken River to feed on a wide range of food organisms of a particular type has been noted. The absence of host specificity would increase their chances of survival in a community which is characterised by a diverse flora, and which is subjected to extreme seasonal fluctuations of environmental conditions. The general predominance of a wide host tolerance suggests that food specificity may not have a critical influence on the distribution pattern of most nematodes.

TABLE 21: Effect of temperature on hatching time (in days) of eggs of nematode species considered in biological studies.

Species	Temperature °C				
	5	10	15	20	25
A. neozelandicus	0	25-26	13-17	6-7	5-7
T. rikus	0	22-28	9-11	5-6	4-5
A. bicaudatus	0	14½-17	7½-9	3-3½	2-3½
S. demani	-	-	-	4½-5	-
D. durus	-	-	-	5½-6	-
A. paraamylovorus	0	0	21-28	10-12	10-14
Tylencholaimus sp.	0	30-34	15-20	9-12	7-10
Acrobeloides sp.	0	19-25	9-11	3-5	4-5

5. DISTRIBUTION

5.1 INTRODUCTION

Populations of living organisms are rarely distributed at random over the space available to them. More frequently, they exhibit contagious distribution patterns characterized by clumping or aggregation of individuals into nodes of high density. Contagious distributions are typical of most species of soil animals (Hughes, 1962).

In reporting a non-random and non-normal distribution of nematodes in British moorland soils, Banage (1966), demonstrated that nematode populations may also exhibit aggregation. Similarly, Zuckerman, Khera and Pierce (1964) found that variations of up to 500% were common for nematodes in upper layer samples taken 15cm apart from cranberry bogs.

Departures from randomness present problems in the quantitative estimation of nematode populations and in the interpretation of the observed variation. Many statistical procedures are of doubtful validity when the assumption of random occurrence of organisms in sample counts does not hold. Data from such populations must be considered using distribution-free tests (Bradley, 1968) or parametric methods after transformation of the data to a different scale so that the transformed variates meet the assumptions of the analysis (Sokal and Rohlf, 1969). It follows that knowledge of the distribution pattern is a pre-requisite to considerations of population dynamics. The present section was designed to investigate the horizontal and vertical distribution of the nematode fauna at Broken River, and thence to consider the number of samples

required to detect seasonal fluctuations with reasonable accuracy.

Thornton (1960a, 1960b) and his co-worker (di Menna, 1960; Stout, 1960a; Ross, 1960) assessed the soil micro-organisms of both cultivated and uncultivated land at Broken River. The examination of samples to investigate the nematode distribution afforded an opportunity to make a similar comparison between improved and unimproved grassland for nematodes.

5.2 ECOLOGICAL CLASSIFICATION OF NEMATODES

The general diversity of the nematode fauna is expressed in the large number of species occupying a wide range of micro-habitats which may be found over small areas of soil. Yeates (1968b) is of the opinion that because nematodes are ecologically diversified "analysis of any variation must be made for individual species". In a contagiously distributed fauna rich in species, such an approach would appear to require large numbers of samples. However, Banage (1964) considered that the role of nematodes in the biology of the soil can be evaluated using ecological feeding groups, that is, by considering functionally related species as a unit. Results of samples from the Broken River fauna were subjected to statistical analysis to determine the practicability of both points of view.

Information on feeding habits of the most commonly occurring nematodes in the fauna were reported in Chapter 3. On the basis of these results, and the assignation of nematodes to feeding groups by previous workers (Neilsen, 1949; Banage, 1963; Szczygiel, 1966; Yeates, 1967), together with the supplementary information given by Hyman, (1951), Winslow (1960) and Goodey (1963), nematodes were placed in ecological feeding categories (Table 22).

Banage (1963) and Yeates (1967) included mycophagous

TABLE 22: Classification of the Broken River nematode fauna into ecological feeding groups.

Higher plant feeders

Aglenchus neozelandicus
Cricenomoides sp.
Cephalenchus tahus
Helicotylenchus sp.
Paratylenchus projectus
Pratylenchus penetrans
Tylenchorhynchus sp.
Tylenchus maiakus

Filamentous feeders

Aphelenchoides bicaudatus
Aphelenchoides sp.
Bursephalenchus sp.
Deladenus durus
Ditylenchus spp.
Nothotylenchus sp.
Tylenchus rikus
Tylenchus sp.
Tylencholaimellus montanus
Tylencholaimellus sp.
Tylencholaimus sp.

Bacterial feeders

Acrobeloides sp.
Cephalobus sp.
Chiloplacus sp.
Cervidellis spp.
Eucephalobus sp.
Mesorhabditis spp.
Rhabditis sp.
Diploscapter sp.
Diploscapteridae
Pelodera sp.
Monhystera sp.
Anaplectus granulosis
Plectus spp.
Rhabdolaimus sp.
Wilsonema otophorum

Miscellaneous feeders

Aporcelaimellus paraamylovorus
Aporcelaimus spp.
Eudorylaimus sp.
Dorylaimus sp.
Labronema sp.

Predators

Brachonchulus sp.
Mononchus spp.
Mylonchulus sp.
Nygolaimus (*Paravulvus*) sp.
Seinura demani

Unknown

Diphtherophera sp.
Dorylaimellus sp.
Miscellaneous Dorylaimida (6 spp.)
Bastiana sp.
Prismatolaimus sp.
Chromodorida sp.
Genus et sp. indent.

nematodes in their plant feeding category. The splitting of this group into 'higher plant feeders' corresponding to Nielsen's (1949) root feeders, and Szczygiel's (1966) plant parasites was considered desirable on two counts: firstly plant parasitic nematodes are the most economically significant group and knowledge of their population ecology is important in interpreting plant disease outbreaks; secondly, the two categories are considered to have different ecological functions. Hence the group called 'higher plant feeders' includes only obligate higher plant feeding forms. The 'filamentous feeder' group encompasses obligate mycophagous nematodes (e.g. Tylenchus rikus; Tylencholaimus sp.) and those species with the capacity to feed on filamentous algae, or higher plant cells (usually with a preference for root hairs). Most of the species included in this category did not penetrate higher plants, but populations of some (e.g. A. bicaudatus), were established on seedlings grown in water agar, on algae or fungi (see Chapter 3), and these species may warrant splitting into a further group of facultative plant parasites. However, the low frequency of occurrence of the animals did not justify the subdivision in the present study. The term 'filamentous feeders' is not intended to exclude species which have the capacity to feed on epidermal plant cells or non-filamentous algae, but seems to best describe the predominant feeding habit of a group of nematodes that feed mostly on the lower forms of plant life. The group does not include bacterial feeding nematodes. Folowing Yeates (1967) terminology, bacterial feeding nematodes are separated from Banage's (1963) microbial feeder group. The miscellaneous feeder category proposed by Banage (1963) is retained, although not all Dorylaimoidea are included. Yeates (1967) called this group microherbivorous and

included many species for which the food source was uncertain, acknowledging that some may have been fungus feeders. In view of current knowledge 'miscellaneous feeders' are defined as nematodes which may feed on plant and animal and possibly microbial material (e.g. A. paraamylovorus). The fifth group of predacious species includes known obligate predators (e.g. Seinura demani) and those species for which evidence indicates a predominantly predacious habit (e.g. Nygolaimus sp., and Mononchus sp.).

5.3 HORIZONTAL DISTRIBUTION

Any consideration of distribution is closely related to the extent of the area to be sampled, to the homogeneity within that area, the size of the sampling unit and the number of samples taken. A population of soil animals randomly distributed over a large area may appear clumped when sampled from a small area or vice versa, or, the distribution may be under dispersed over both large and small areas. To accomodate these possibilities two patterns of sampling were adopted: in the first, a series of closely spaced samples was taken from improved pasture and about 200 metres distant a similar series was taken from unimproved grassland; in the second, stratification was included in a series of samples from plots of about 400 square metres on the two sites. Sample unit size was defined in Chapter 2. With a soil core of 2.5cm diameter to a depth of 7.5cm nematode numbers in the order of 1,000 to 6,000 animals per unit sample were obtained.

5.3.A Transect samples

METHOD

Fescue tussocks dominate the macro-flora of the study area. Scott(1961) showed that the density of inter-tussock plants decreased with increased distance from the base of the tussocks. Hence it seemed that population gradients of nematodes related to the position of the tussocks might also exist.

To investigate the spatial relationships between aggregates over a small area, a sequential series of samples was taken from both improved and unimproved soil, with fescue tussocks about 90cm apart as the terminal sample sites. Soil cores were removed from within the tussocks and from stations at 15cm intervals along a transect over the inter-tussock area between them. Samples were processed and the nematodes counted as described in Chapter 2. The specific composition of the plant cover was determined for individual cores.

Results were analysed to investigate the effects of grassland improvement on the faunal density and composition and to characterize the distribution of nematodes between closely spaced tussocks.

RESULTS AND DISCUSSION

Sixty-nine nematode species were identified from the total of 16 samples (Appendix IV A-B). Forty-seven species were recognized from the unimproved grassland and 52 species from the improved grassland. Of these, 30 species occurred in samples from both sites. The most frequently occurring nematodes were, with a few exceptions, common to both areas.

5.3.A.I Effect of pasture improvement on nematode density

The Mann-Whitney U-test (Sokal and Rohlf, 1969) was used to test the equality of location of the two distributions for total numbers of nematodes and numbers in the respective ecological feeding groups. The two samples differed significantly in the distribution of total nematodes ($0.05 > P > 0.02$), plant feeders ($0.05 > P > 0.02$), bacterial feeders ($0.2 > P > 0.1$) and miscellaneous feeders ($0.05 > P > 0.02$), with samples from improved pasture containing the greatest number of animals. It is apparent that pasture improvement has resulted in a general increase in the nematode fauna but the increase is not uniform throughout the ecological groups within the fauna.

5.3.A.II Effect of tussock position on distribution of nematodes in the inter-tussock zone

Data for total nematodes and the ecological feeding groups were arranged in 2x8 contingency tables and independence or association of frequencies tested using a G test (Sokal and Rohlf, 1969). To test for homogeneity between sample stations all combinations of rows and columns were analysed using the sum of squares STP proceedure (Sokal and Rohlf, 1969). The results in Table 23 show that the frequency of total nematodes, plant feeders, filamentous feeders, bacterial feeders, and predators is dependent on whether the samples were taken from improved or unimproved soil. Tests on sub-set combinations demonstrated the independence of frequencies from the position of the sample on the transect. That is, there were no apparent positive or negative gradients from the tussock plants, and the distribution of nematodes from sample stations between tussocks was independent of the tussock position.

TABLE 23: Test of independence of frequencies of nematodes from transect samples from the inter-tussock zone of improved and unimproved tussock grassland.

	G for total set
Total nematode	46.50
Plant feeders	39.46
Filamentous feeders	31.40
Bacterial feeders	24.45
Miscellaneous feeders	22.54
Predators	19.08

X^2 (7df)
0.05 = 14.07

All sets significant

5.3.A.III Microdistribution

Despite the small number of cores, and the fact that they were taken a long a single transect in a two-dimensional system, there is evidence of a series of gradients leading to nodes of higher density which are apparent at the total population and ecological feeding group levels, and which are also indicated for some of the most commonly occurring species (Figures 42 and 43). The size of the aggregate is difficult to determine unless a complete enumeration programme is undertaken (Nielsen, 1954; Peachey, 1963) but the trends indicate that specific aggregates of at least 45cm diameter occur (e.g. A. neozelandicus from samples six, seven and eight of both sites).

FIGURE 42. Numbers of nematodes per ml of soil from transect samples taken on improved tussock grassland. Sequential samples (1-8) indicated by plant species.

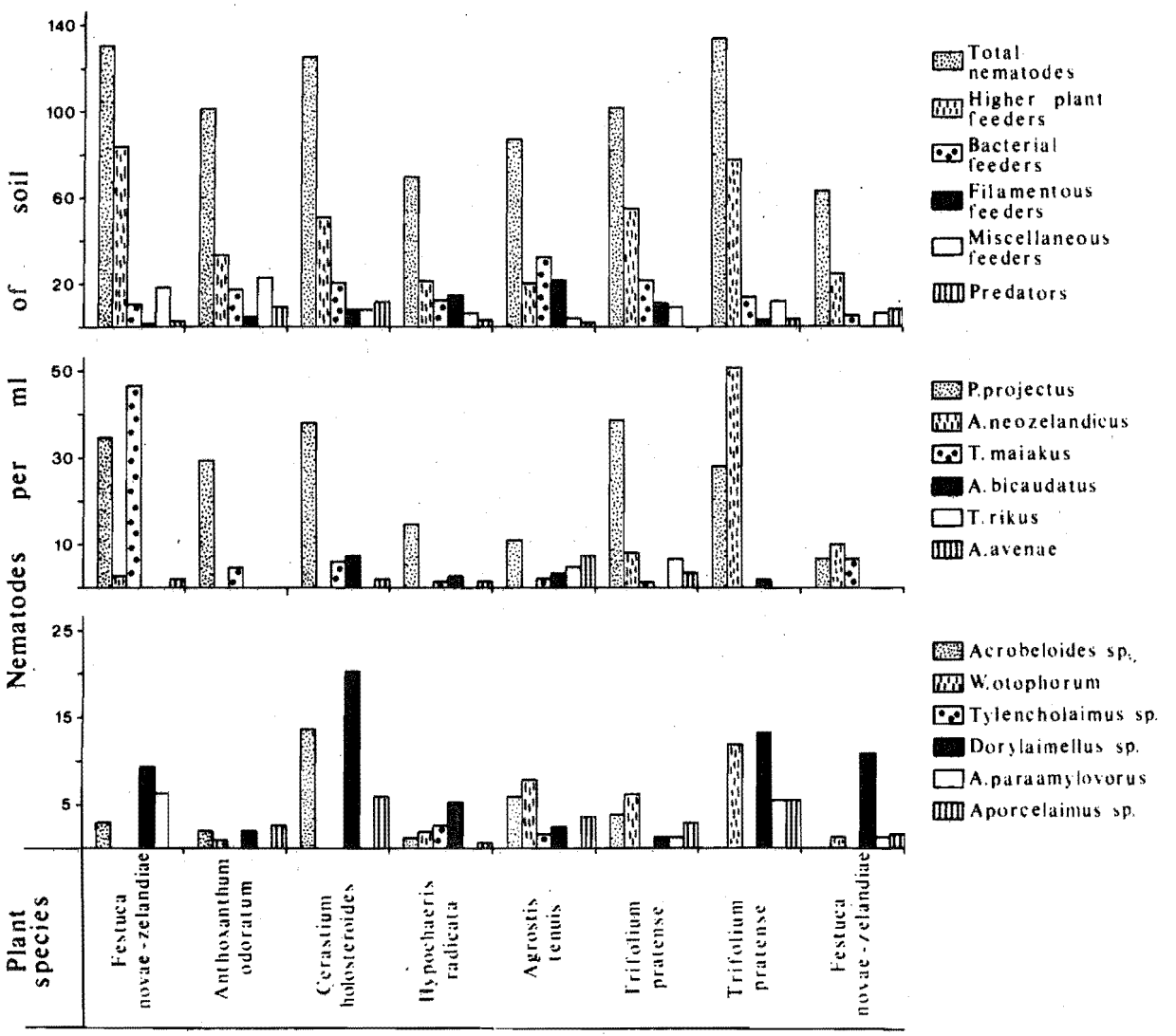
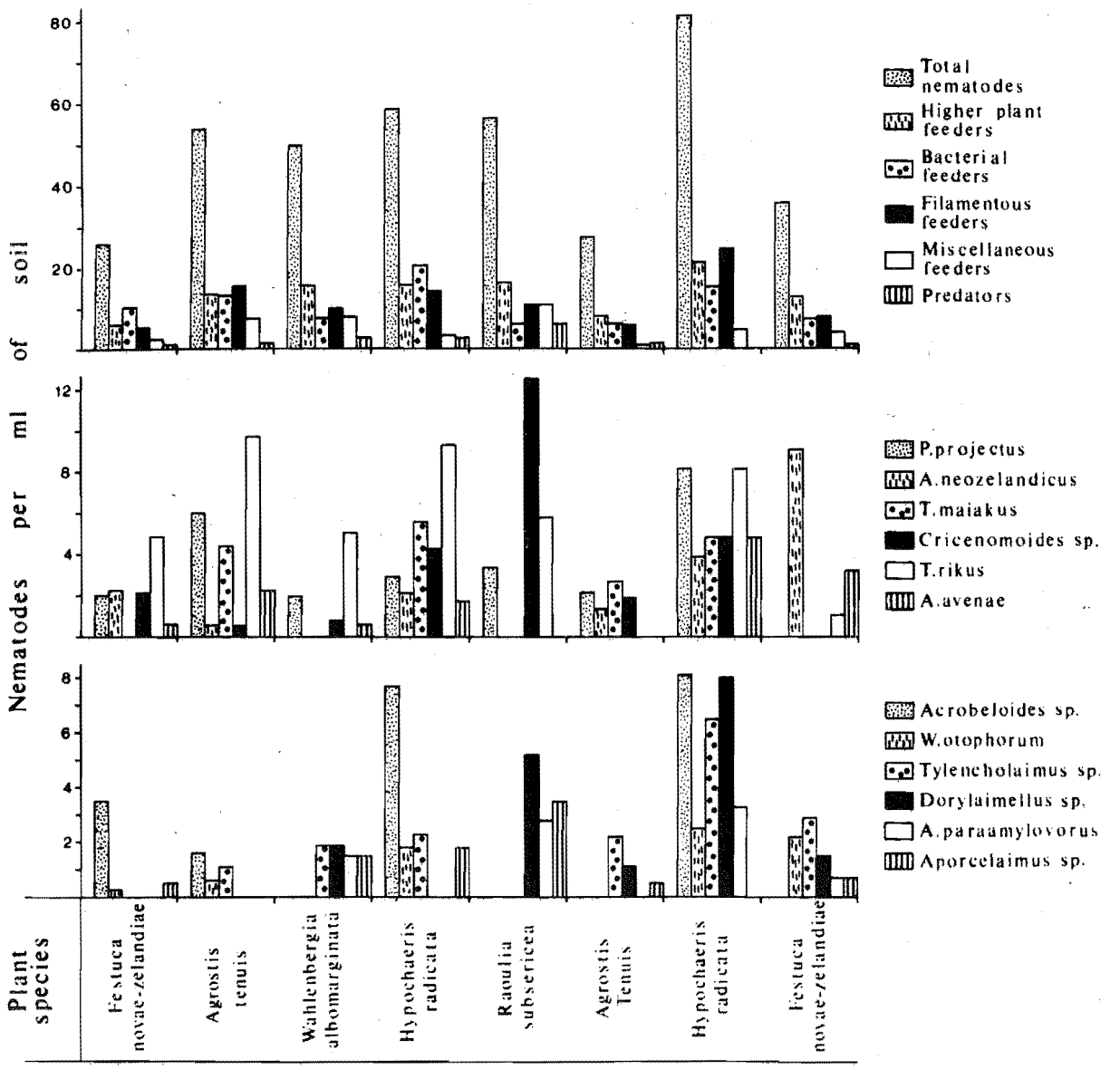

Nematodes per ml of soil
140
100
60
20
50
30
10
25
15
5
Total nematodes
Higher plant feeders
Bacterial feeders
Filamentous feeders
Miscellaneous feeders
Predators
P.projectus
A.neozelandicus
T. maiakus
A. bicaudatus
T. rikus
A.avenae
Acrobeloides sp.
W.otophorum
Tylencholaimus sp.
Dorylaimellus sp.
A.paraamylovorus
Aporcelaimus sp.
Plant species
Festuca novae-zelandiae
Anthoxanthum odoratum
Cerastium holosteroides
Hypochaeris radicata
Agrostis tenuis
Trifolium pratense
Trifolium pratense
Festuca novae-zelandiae

FIGURE 43. Numbers of nematodes per ml of soil from transect samples taken on unimproved tussock grassland. Sequential samples (1-8) indicated by plant species.


Nematodes per ml of soil
80
60
40
20
Total nematodes
Higher plant feeders
Bacterial feeders
Filamentous feeders
Miscellaneous feeders
Predators
12
8
4
P.projectus
A.neozelandicus
T.maiakus
Cricenomoides sp.
T.rikus
A.avenae
8
6
4
2
Acrobeloides sp.
W.otophorum
Tylencholaimus sp.
Dorylaimellus sp.
A.paraamylovorus
Aporcelaimus sp.
Plant species
Festuca novae-zelandiae
Agrostis tenuis
Wahlenbergia albomarginata
Hypochaeris radicata
Raoulia subsericea
Agrostis Tenuis
Hypochaeris radicata
Festuca novae-zelandiae

5.3.B Tussock rhizosphere samples

In discussing the micro-distribution of an aggregated enchytraeid population Nielsen (1954) noted the advantages of stratification of the sample site. He suggested that stratification based on a biological feature of the area or even on a purely geometrical basis would offer advantages over non-stratified areas "when the (invisible) soil fauna is being sampled". To reduce variation, stratification based on a single plant species as the "biological feature" was incorporated in the present sampling series.

Tussocks were chosen as the sample environment because of their dominant position in the community and because they provided a reasonably large rhizosphere which ensured a standard reference unit.

METHOD

To test the reliability of results from samples from the rhizosphere of fescue tussocks, cores were taken from the centre and the perimeter of plants of good and poor condition growing on improved and unimproved tussock grassland (Figure 43A). Plants were considered to be in poor condition when degeneration and death of tillers was evident over part of the crown. Two plants were sampled for each category. The sampling stations were determined by random numbers and no two stations were less than two metres apart.

RESULTS AND DISCUSSION

A total of 68 nematode species were recognised. Of 41 species isolated from tussocks grown on improved soil, and 48 species from tussocks grown on unimproved soil,

FIGURE 43A. Design of experiment to investigate the effects of stratification on the variability of the nematode fauna expressed in a number of samples.

IMPROVED FESCUE TUSSOCK GRASSLAND

- GOOD GROWTH CONDITION
  - CENTRE
  - PERIMETER
- POOR GROWTH CONDITION
  - CENTRE
  - PERIMETER

UNIMPROVED FESCUE TUSSOCK GRASSLAND

- GOOD GROWTH CONDITION
  - CENTRE
  - PERIMETER
- POOR GROWTH CONDITION
  - CENTRE
  - PERIMETER

31 were common to both sites.

5.3.B.I Homogeneity of variances

Homogeneity of variances (homoscedasticity) is an important fundamental assumption for several statistical tests, including analysis of variance. In view of the heterogeneity in the Broken River fauna this assumption was tested before proceeding to parametric methods of analysis. Bartlett's test of homogeneity of variances and the variance ratio F_{max}-test (Sokal and Rohlf, 1969), were used to test data for total nematodes, ecological feeding groups and the most common plant feeding species. Because of the aggregation evident in transect samples data were transformed to log (x+ 0.01)(see Southwood, 1968, page 11).

The results presented in Table 24 show that the value of X^2 for most of the components tested is non-significant. Homoscedasticity is corrobated by the results of the variance ratio F_{max}-test (Table 24).

5.3.B.II Analysis of variance

Variation of the results associated with 'level of nutrition' (plants growing in improved and unimproved tussock grassland) 'state of growth', 'position within plant', or interactions between these effects were investigated using four factor factorial analysis of variance without replication (Sokal and Rohlf, 1969), on log transformed data. The 'replicate' or second sample from each category was entered into the analysis as a random factor.

Agricultural improvement significantly increased ($P < 0.01$) the total number of nematodes harvested, and numbers were greater in the rhizosphere of plants classed as growing in good condition (Table 25). Numbers of plant

TABLE 24: Test for homogeneity of variances of nematode numbers per ml of soil from tussock (*F. novae-zelandiae*) rhizosphere samples

	Adj. X^2	FS
Total nematodes	0.5236	1.7683
Plant feeders	0.0391	1.1676
Filamentous feeders	6.0933*	8.0405
Bacterial feeders	0.0581	1.2077
Miscellaneous feeders	6.0904*	8.0360
Predators	0.7021	1.9377

X^2(1df)	F_{max}(6.7df)
0.05=3.841*	0.05=15.0*
0.01=6.635**	0.01=27.0**

feeding nematodes were significantly larger ($P < 0.01$) under tussocks growing in improved soil and greater ($P < 0.01$) under plants in good condition (Table 26). The number of bacterial feeders was significantly increased ($P < 0.05$) on improved soil (Table 27), but there was no main factor effects or interaction effects evident in the results for filamentous feeders, miscellaneous feeders, or predators (Tables 28-30). Bacterial species comprise species of the orders Rhabditida and Araeolaimida (Table 22). Analysis of data for each of these groups indicates that the increase in bacterial feeders is reflected in an increase ($P < 0.01$) in the numbers of Rhabditida, rather than Araeolaimida (Tables 31 and 32).

A degenerative condition was observed in some F. novae-zelandiae plants at Broken River. Zotov (1938) noted the depletion of tussocks in patches and suggested that fungus attack was involved. The lack of association between any one species of plant feeding nematode with tussocks exhibiting poor growth, and the reduction in numbers of plant feeding species in the rhizosphere of depleted plants, indicates that nematodes are not the primary incitants.

Fescue tussock plants frequently cover an area of 150 to 400 square centimetres at the base. It was considered that if the influence of plant species on the nematode fauna is small, contagious distribution patterns within tussock plants may occur; or on the other hand, if the plant species effect is real, the proximity of the inter-tussock species could influence the nematode composition of the peripheral within-tussock samples. The analysis shows that the micro-distribution of nematodes is relatively constant within the F. novae-zelandiae rhizosphere.

TABLE 25: Analysis of variance table for total numbers of nematodes per ml of soil from tussock (F. novae-zelandiae) rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0023	1	0.0023	0.2396	NS
C State of growth	0.0555	1	0.0555	5.7812	*
D Level of nutrition	0.7188	1	0.7188	74.8750	**
B x C	0.0209	1	0.0209	2.1771	NS
B x D	0.0000	1	0.0000	0.0000	NS
C x D	0.0003	1	0.0003	0.0312	NS
B x C x D	0.0055	1	0.0055	0.5729	NS
Residual	0.0675	7	0.0096		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 26: Analysis of variance table for numbers of plant feeding nematodes per ml of soil from tussock (F. novae-zelandiae) rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0217	1	0.0217	1.9203	NS
C	0.1714	1	0.1714	15.1681	**
D Level of nutrition	1.0460	1	1.0460	92.5664	**
B x C	0.0057	1	0.0057	0.5044	NS
B x D	0.0028	1	0.0028	0.2478	NS
C x D	0.0021	1	0.0021	0.1858	NS
B x C x D	0.0044	1	0.0044	0.3893	NS
Residual	0.0792	7	0.0113		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 27: Analysis of variance table for numbers of bacterial feeding nematodes per ml of soil from tussock (F. novae-zelandiae)rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0014	1	0.0014	0.0522	NS
C State of growth	0.0002	1	0.0002	0.0075	NS
D Level of nutrition	0.2759	1	0.2759	10.2948	*
B x C	0.0047	1	0.0047	0.1753	NS
B x D	0.0006	1	0.0006	0.0224	NS
C x D	0.0115	1	0.0115	0.0522	NS
B x C x D	0.0040	1	0.0040	0.1492	NS
Residual	0.1877	7	0.0268		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 28: Analysis of variance table for numbers of filamentous feeding nematodes per ml of soil from tussock (F.novae-zelandiae) rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0000	1	0.0000	0.0000	NS
C State of growth	0.0008	1	0.0008	0.0439	NS
D Level of nutrition	0.0007	1	0.0007	0.0387	NS
B x C	0.0000	1	0.0000	0.0000	NS
B x D	0.0158	1	0.0158	0.8681	NS
C x D	0.0006	1	0.0006	0.0330	NS
B x C x D	0.0013	1	0.0013	0.0714	NS
Residual	0.1272	7	0.0182		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 29: Analysis of variance table for numbers of miscellaneous feeding nematodes per ml of soil from tussock (*F. novae-zelandiae*) rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0146	1	0.0146	0.8391	NS
C State of growth	0.0281	1	0.0281	1.6149	NS
D Level of nutrition	0.0043	1	0.0043	0.2471	NS
B x C	0.0061	1	0.0061	0.3506	NS
B x D	0.0010	1	0.0010	0.0575	NS
C x D	0.0125	1	0.0125	0.7184	NS
B x C x D	0.0067	1	0.0067	0.3850	NS
Residual	0.1216	7	0.0174		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 30: Analysis of variance table for numbers of predatory nematodes per ml of soil from tussock (*F. novae-zelandiae*) rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0298	1	0.0298	1.4975	NS
C State of growth	0.0026	1	0.0026	0.1306	NS
D Level of nutrition	0.0250	1	0.0250	1.2562	NS
B x C	0.0299	1	0.0299	1.5025	NS
B x D	0.0552	1	0.0552	2.7739	NS
C x D	0.0079	1	0.0079	0.3970	NS
B x C x D	0.0063	1	0.0063	0.3166	NS
Residual	0.1391	7	0.0199		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 31: Analysis of variance table for numbers of Rhabditida per ml of soil from tussock (*F. novae-zelandiae*) rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0003	1	0.0003	0.0173	NS
C State of growth	0.0009	1	0.0009	1.2370	NS
D Level of nutrition	0.3333	1	0.3333	19.2659	**
B x C	0.0004	1	0.0004	0.0231	NS
B x D	0.0001	1	0.0001	0.0058	NS
C x D	0.0214	1	0.0214	1.2370	NS
B x C x D	0.0008	1	0.0008	0.0462	NS
Residual	0.1213	7	0.0173		

F_s 0.05 = 5.59*
0.01 =12.25**

TABLE 32: Analysis of variance table for numbers of Araeolaimida per ml of soil from tussock (*F. novae-zelandiae*)rhizosphere samples

Source of variation	SS	DF	MS	F	Significance
B Position within plant	0.0120	1	0.0120	0.7100	NS
C State of growth	0.0008	1	0.0008	0.0473	NS
D Level of nutrition	0.0007	1	0.0007	0.0414	NS
B x C	0.0083	1	0.0083	0.4911	NS
B x D	0.0030	1	0.0030	0.1775	NS
C x D	0.0018	1	0.0018	0.1065	NS
B x C x D	0.0044	1	0.0044	0.2603	NS
Residual	0.1181	7	0.0169		

F_s 0.05 = 5.59*
0.01 =12.25**

5.3.B.III Aggregation

Aggregation was tested using Morisita's index of dispersion (Southwood, 1968). The index has the advantage of being independent of the type of distribution and the size of the mean (Morisita, 1962). The first index as described by Southwood (1968) is given by:

$$I_\delta = N \frac{\sum_{i=1}^{N} ni\ (ni - 1)}{\sum x\ (\sum x - 1)} = N \frac{\sum x^2 - \sum x}{(\sum x)^2 - \sum x}$$

where N = total samples, n= numbers in ith sample and $\sum x$ the sum of the number of individuals found in the samples. The significance of the departure from randomness can be tested by calculating F_o (Southwood, 1968):

$$Fo = \frac{I_\delta\ (\sum x - 1) + N - \sum x}{N - 1}$$

and comparing this value with F (from variance ratio tables) where $N_1 = N-1$ and $N_2 = \infty$.

The results presented in Table 33 confirm the contagious nature of the distribution of nematodes at Broken River.

5.3.B. IV Sample number

Data from the tussock rhizosphere samples was analysed to investigate the practicability of maintaining a simultaneous sampling programme from both sites at monthly intervals. The number of samples required to estimate the true mean of the total fauna within $\pm$ 5% with a 5% risk that the error would exceed these limits were calculated using the formula:

$$n = \frac{4\delta^2}{L^2}$$

TABLE 33: Indices of dispersion and the significance of the departure from randomness for nematodes extracted from tussock (*F. novae-zelandiae*) rhizosphere samples

	I	F_o
Total nematodes	1.262	27.340
Higher plant feeders	1.620	31.320
Filamentous feeders	1.072	2.138
Bacterial feeders	1.519	8.740
Miscellaneous feeders	1.303	3.591
Predators	4.794	13.440
P. projectus	2.010	19.900
A. neozelandicus	4.635	58.430
T. maiakus	4.146	49.800
T. rikus	1.576	4.084
A. bicaudatus	2.123	5.096
Tylencholaimus sp.	1.678	3.123
Acrobeloides sp.	1.655	3.908
W. otophorum	1.641	2.582
A. paraamylovorus	3.489	5.115
Dorylaimellus sp.	2.716	6.719

F_s ($N_1 = 15$; $N_2 = \infty$)

0.05 = 1.67

0.01 = 2.04

All sets significant ($P < 0.01$)

where n is the number of samples, Δ^2 is the variance, and L the allowable error (Snedecor and Cochran, 1967). On this basis 85 samples were required for samples from improved tussock, and 64 samples from unimproved tussock. The impracticability of such a comparison was subsequently confirmed by calculations to determine the number of samples required to detect differences between two means in a population of means, using the formula given by Sokal and Rohlf (1969):

$$n \geq 2\left(\frac{\sigma}{\delta}\right)^2 \left\{t_{\alpha[v]} + t_{2(1-P)[v]}\right\}^2$$

where n = the true number of replications; σ true standard deviation; δ the smallest difference that it is desired to detect; v = degrees of freedom of the sample standard deviation (with 'a' groups and n replications per group v = a (n-1)); t = significance level; P = desired probability that a difference will be found to be significant (if it is as small as δ). $t_{\alpha[v]}$ and $t_{2(1-P)[v]}$ = values from the two tailed table with v degrees of freedom and corresponding to probabilities of α and 2 (1-P), respectively.

To be 95% certain of detecting a 10% difference in means of total nematode numbers 95% of the time in a monthly sampling programme for a year, 552 samples would be required on improved tussock grassland and 412 samples on the unimproved site.

5.4 STRATIFIED TUSSOCK SAMPLES

In an attempt to reduce the variability associated with the level of nutrition and the state of growth of plants, further stratification was incorporated in a series

of samples taken only from tussocks growing in good condition on cultivated soil. The cultivated area was chosen because:

i) it provided a greater concentration of F. novae-zelandiae plants per unit area;
ii) of the general uniformity of tussock size and growth habit;
iii) the nematode density per sample was greater on improved than unimproved soil;
iv) phytophagous nematodes constituted a larger proportion of the fauna.

In the previous section, macroscopic appraisal of the above-ground flora was taken as an index of environmental variability. In the present series the plant factor was standardised as far as possible and the variability was assessed in terms of the micro-habitat from which each sample was drawn. Measurements of temperature, moisture, loss on ignition, and soil pH were recorded. In addition, the presence or absence of nematode-trapping fungi was assessed.

METHOD

Two soil cores were drawn from each of 10 F. novae-zelandiae plants. The plants sampled were determined using a table of random numbers. Nematodes were extracted from one core of each pair, and the remaining core was used to determine the environmental parameters.

Soil temperature at each sample station was recorded at a depth of 2.5cm using a mercury-in-glass- thermometer. Moisture was calculated as a percentage of the oven dry weight after drying approximately 10g of soil for 12 hours at 105-110°C (Metson, 1956). A 1:2.5 mixture of soil in distilled water was used for pH determinations after

standing the suspension for 12 hours (Jackson, 1958). Nematode-trapping fungi were isolated by seeding Petri plates containing 15ml of Difco cornmeal agar with approximately 1g of soil from each sample. The dishes were examined at weekly intervals for 14 weeks.

RESULTS AND DISCUSSION

5.4.A Environmental variation

There were no detectable relationships between variation of the environmental parameters measured for each sample and variation of the number of nematodes extracted (Table 34).

5.4.B Aggregation

A significant departure from a random distribution throughout the fauna was demonstrated using Morisita's index of dispersion (Table 35).

5.4.C Number of replications required per sampling period

Using the formula described in section 3.B.IV. it was found that 197 samples would be required to estimate the true mean of the total fauna within $\pm$ 5% risk that the error might exceed these limits; and 641 samples would be required to be 95% certain of detecting a 10% difference in means of total nematodes 95% of the time for a monthly sampling programme over a year.

Taking into account the time required to process and examine each sample and the 'safe' period of storage for samples at $4^{o}C$ (see Chapter 2.3.B.III), 10 samples were considered to be the maximum number practicable for any one period. But by using the 'random sub-sampler' apparatus described in Chapter 2.3.C, to separate four equal aliquots

TABLE 34: Comparison of nematodes per cc of soil from 10 stratified tussock (*F. novae-zelandiae*) plants with measurements of soil moisture, soil temperature, pH, loss on ignition and the presence or absence of nematode-trapping fungi.

	Sample number									
	1	2	3	4	5	6	7	8	9	10
Total nematodes	142.9	156.8	96.0	136.3	88.0	133.3	95.2	80.05	120.3	89.6
Plant feeders	71.5	103.5	38.4	53.1	46.6	44.0	41.9	41.6	46.9	28.7
Filamentous feeders	2.9	14.1	9.6	9.5	9.7	10.7	9.5	9.6	19.2	28.7
Bacterial feeders	60.0	21.9	25.9	60.0	21.1	30.0	20.0	21.6	30.1	16.1
Miscellaneous feeders	4.3	17.2	12.5	8.2	7.9	17.33	14.3	2.4	8.4	8.1
Predators	0.0	0.0	1.9	0.0	0.0	10.7	0.9	1.6	3.6	2.7
Soil moisture (%)	36.7	32.0	30.2	32.4	35.8	38.1	33.8	35.3	33.6	35.1
Soil temperature °C	18.0	18.5	17.2	17.5	18.0	18.0	17.5	17.5	17.0	18.0
pH	5.7	6.1	5.8	5.9	5.2	6.0	6.1	5.7	5.9	6.0
Loss on ignition	14.8	10.1	11.8	10.1	14.4	13.3	11.0	13.2	11.8	14.4
Presence of nematode-trapping fungi (+)	-	-	-	-	+	+	+	-	-	-

TABLE 35: Indices of dispersion and significance of the departure from randomness for nematodes extracted from stratified tussock (*F. novae-zelandiae*) samples.

	I	F_o	Significance
Total nematodes	1.044	6.517	**
Higher plant feeders	1.136	8.800	**
Filamentous feeders	1.223	4.032	**
Bacterial feeders	1.221	4.032	**
Miscellaneous feeders	1.441	2.595	**
Predators	2.732	4.932	**
P. projectus	3.332	35.870	**
A. neozelandicus	1.944	19.800	**
T. maiakus	1.808	17.961	**
T. rikus	1.563	3.536	**
A. bicaudatus	1.400	1.980	*
Tylencholaimus sp.	2.281	6.158	**
Acrobeloides sp.	1.500	3.787	**
W. otophorum	3.082	7.024	**
A. paraamyloverus	1.253	2.345	*
Dorylaimellus sp.	2.763	7.094	**

F_s ($N_1 = 9$; $N_2 = \infty$)
0.05 = 1.88*
0.01 = 2.41**

TABLE 36: Expected detectable differences between means with 95% confidence limits ($P < 0.05$) based on the results of stratified tussock samples

	Detectable difference between two means %	Number of replicates (8 sampling periods)
Total nematodes	28	40.86
Higher plant feeders	31	41.38
Filamentous feeders	43	41.26
Bacterial feeders	40	40.74
Miscellaneous feeders	38	39.70
Predators	114	41.34
P. projectus	122	40.10
A. neozelandicus	80	40.16
T. maiakus	75	39.30
T. rikus	70	40.80
A. bicaudatus	80	40.61
Tylencholaimus sp.	97	41.38
Acrobeloides sp.	65	40.30
W. otophorum	122	40.86
A. paraamylovorus	53	39.85
Dorylaimellus sp.	114	39.83

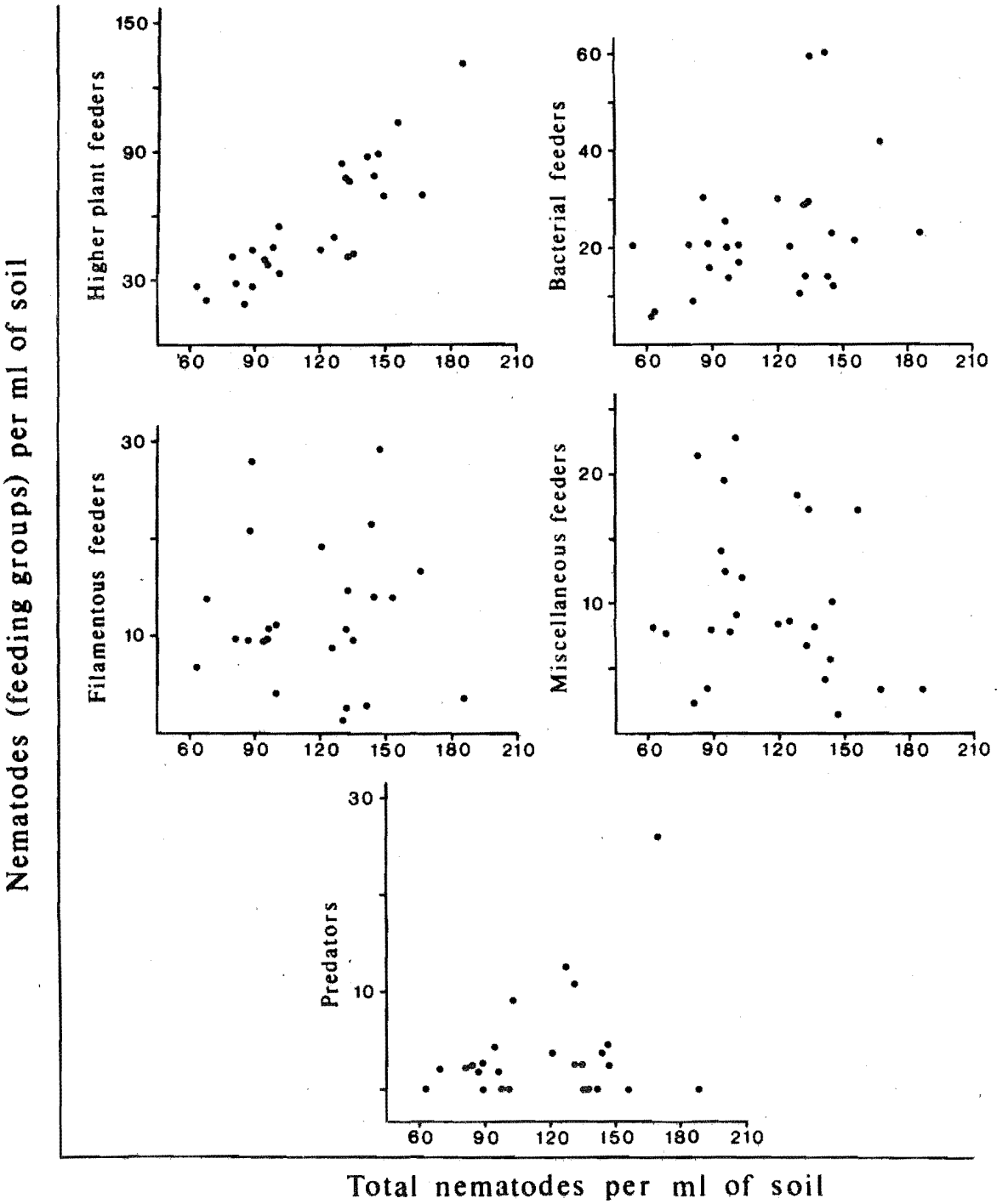
it was possible to increase the number of field samples to 40 without appreciably increasing the amount of laboratory work involved. Taking n=40 for each of eight sampling periods over a year, to calculate V and substituting for different values of δ the differences detectable between means with 95% confidence at the 5% level of significance were calculated (Table 36). It is apparent that comparisons at the species level must be regarded with reservation.

5.5 BIOLOGICAL SIGNIFICANCE OF AGGREGATION

In a nematode fauna comprising several species, the presence of aggregation suggests the clumping of more than one species at a given centre, or that aggregations overlap. In soil samples from Broken River, many species of nematodes representing several ecological feeding groups were present. By grouping the results of the three sampling series from improved tussock grassland and plotting numbers per ml of soil for different 'ecological levels' of the fauna against the total faunal number per ml (Figure 44), it is apparent that nodes of high total density do not necessarily result from a uniform increase throughout the fauna. Both clumping and over-lapping of aggregates are indicated. The large contribution of higher plant feeding nematodes to the faunal variation is reflected in the complementary distribution of higher plant feeders and the total fauna.

With the multiplicity of factors operating in the soil, and the ecological diversity of the nematode fauna, a contagious distribution pattern is not unexpected. Several potentially aggregative mechanisms are demonstrated in considerations of the biology of nematodes in Chapter 4. For example, host attraction occurred in plant and filamentous feeding forms, clumping of egg masses was observed,

FIGURE 44. Relationship between numbers of nematodes in ecological feeding groups per ml of soil to total nematodes per ml of soil.


Nematodes (feeding groups) per ml of soil
Higher plant feeders
150
90
30
60 90 120 150 180 210
Bacterial feeders
60
40
20
60 90 120 150 180 210
Filamentous feeders
30
10
60 90 120 150 180 210
Miscellaneous feeders
20
10
60 90 120 150 180 210
Predators
30
10
60 90 120 150 180 210
Total nematodes per ml of soil

sex attraction was demonstrated in one species, predator-prey interactions occurred, and the presence of nematode parasites was recorded. While these factors must be important in the population a great deal of the patchiness can be undoubtedly by explained in terms of local differences in micro-habitat suitability, and competition between animals for available resources.

Clark, Geier, Hughes and Morris (1967) reviewed current theories on population ecology. It is apparent from the many views discussed (e.g. Nicholson, Andrewartha and Birch, Milne ....) that shortage, inacessability, or abundance of resources, whether induced by environmental changes, competition, or particular biological characteristics of individuals, play a major role in determining population numbers. Clearly, local variations in the soil environment would affect the relative proportions of functionally unrelated groups in samples, and through density dependent effects, influence the numbers of animals within these groups. But unless some additional mechanism is operative one could reasonable expect dispersal from various loci to result in uniformity of 'within ecological groups' specific composition of samples from small areas. Even if outward migration of nematode populations is restricted to about one or two metres per year (Wallace, 1963), over a period of uninterrupted spread from several centres local interspersion of specific aggregates would result. Comparison of the densities and frequency of occurrences of the three most abundant higher plant feeding nematodes in the series of samples from improved grassland shows that such a situation is not reached (Table 37). Two explanations seem possible; firstly, that a high degree of host specificity occurs, or secondly, that interspecific competition to the extent of suppression of one species by another is operative.

TABLE 37: Comparison of frequency of occurrence and density of higher plant feeding nematodes in improved tussock grassland

Tussock rhizosphere samples	Nematodes per ml of soil							
	1	2	3	4	5	6	7	8
P. projectus	33.49	2.86	39.68	55.26	20.05	38.28	41.85	26.87
A. neozelandicus	93.04	85.89	17.63	10.18	0.00	0.00	0.00	0.00
T. maiakus	5.58	0.00	11.76	5.82	54.81	29.96	1.95	2.47
Other species	0.00	0.00	20.59	7.26	1.34	0.00	1.95	0.27

Transect samples	Nematodes per ml of soil							
	1	2	3	4	5	6	7	8
P. projectus	34.15	29.43	37.96	14.63	11.26	38.63	28.05	6.99
A. neozelandicus	2.63	0.00	0.00	0.00	0.00	9.30	50.69	10.81
T. maiakus	47.28	4.06	5.06	0.70	1.73	1.02	0.00	6.99
Other species	0.00	0.00	7.59	5.48	5.19	1.02	0.00	0.00

Stratified tussock samples	Nematodes per ml of soil									
	1	2	3	4	5	6	7	8	9	10
P. projectus	0.00	45.47	32.36	6.81	6.16	4.00	3.81	0.00	0.00	3.58
A. neozelandicus	47.17	0.00	0.00	0.00	40.48	0.00	38.08	8.00	24.05	22.40
T. maiakus	18.58	40.77	5.76	44.97	0.00	40.00	0.00	31.22	6.01	2.69
Other species	5.72	17.25	0.00	1.36	0.00	0.00	0.00	2.41	16.81	0.67

Host specificity is not considered to be of major importance. Wide host ranges were shown for two of the higher plant feeding species concerned (P. projectus and A. neozelandicus) and indeed for species representing other ecological feeding groups (see Chapter 3). In addition, two thirds of the samples were confined to the rhizosphere of fescue tussock plants. Interspecific suppression has been demonstrated between species of plant feeding nematodes (Johnson, 1968). In multiple inoculations with several species on a range of hosts Johnson showed that in most experiments the presence of one nematode species adversely affected the reproduction of others. Interspecific competition would promote aggregation in higher plant feeding nematodes and may possibly influence aggregation of species within other ecological feeding groups.

It is apparent that no one factor can be isolated from another in considering aggregation of nematode populations. With the complicated interactions occurring it is probable that the fauna is in a state of flux; localised blooms continually appearing and disappearing. Because of the diversity of the nematode community, the effect of severe oscillations of species or ecologically related species on the faunal density could be buffered by the 'short-term' relative stability of ecologically unrelated groups. Such a system could well explain the disproportionate representation which occurred between nematodes of different feeding groups in samples of fescue tussock grassland soil.

5.6 VERTICAL DISTRIBUTION

METHOD

Two series of samples were taken. Pits approximately one metre square were dug to a depth of about 70cm. Soil

cores were drawn horizontally from the cut face at measured distances from the surface, from the rhizosphere of a fescue tussock and, from a position in the inter-tussock zone approximately 25cm away. In the first series samples were taken at datum (0.0-2.5cm), 7.5-10.0cm, 15.0-17.5cm, 30-0-32.5cm, and 60-0-62.5cm, and in the second series taken two months later, from datum, 4.0-6.5cm, 15.0-17.5cm, 30-0-32.5cm. The final sample retained for elutriation was equivalant to about 37.5ml of soil.

RESULTS AND DISCUSSION

The greatest density of nematodes occurred in the top 10-15cm of soil, with maxima near the surface (Figure 45). In the first series, higher plant feeding nematodes were concentrated in the 7.5-10.0cm sample but were more uniformly spread in samples taken two months later (Table 38). Two interpretations seem possible; that aggregation also occurs in the vertical plane, or that seasonal environmental effects cause a shift in maxima.

It is apparent that estimates of the total fauna based on cores to a depth of 7.5cm understate the actual situation. Because there is no apparent stratification of ecological feeding groups in the vertical plane (Table 38) the samples are considered to be a valid estimate of the relative proportions of the groups in the nematode fauna.

5.7 GENERAL DISCUSSION

The distribution of nematodes in fescue tussock soil was shown to be contagious. Overall density varied considerably in random samples from an area of 20 metres square, and the contagious distribution was retained in samples 15cm apart. Total aggregation was superimposed on aggregation of ecological feeding groups. Aggregate size was

FIGURE 45. Variation in total nematode numbers per ml of soil with depth of sampling in the horizontal plane.

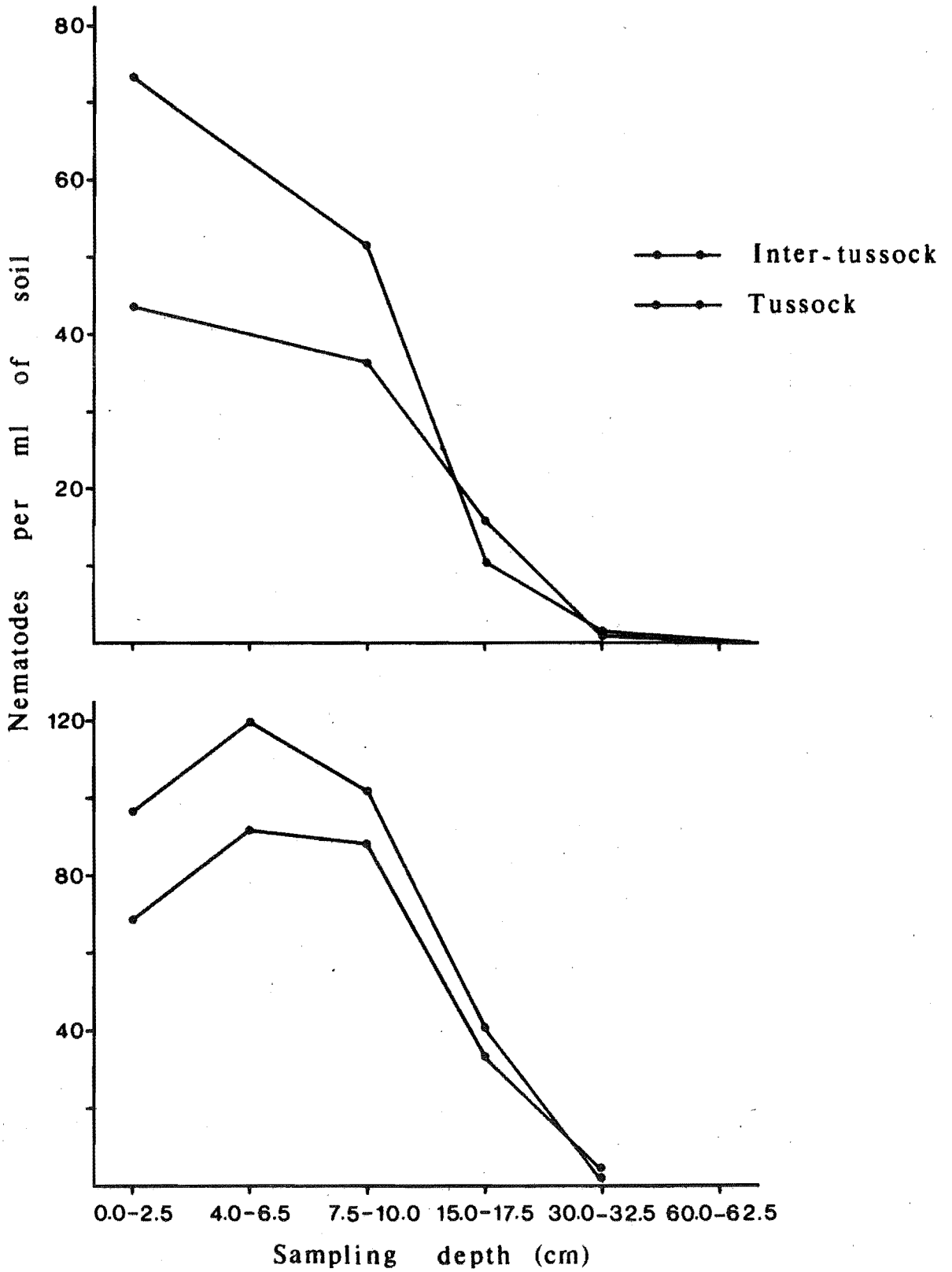

Nematodes per ml of soil
80
60
40
20
120
80
40
Inter-tussock
Tussock
0.0-2.5
4.0-6.5
7.5-10.0
15.0-17.5
30.0-32.5
60.0-62.5
Sampling depth (cm)

TABLE 38: Vertical distribution of ecological feeding groups in two series of samples taken from the tussock and inter-tussock zones.

Ecological feeding group	Location of sample	Numbers of nematodes per cc of soil at depths of... (cm)					
		0.0-2.5	4.0-6.5	7.5-10.0	15.0-17.5	30.0-32.5	60.0-62.5
Higher plant	Inter-tussock	8.9		28.6	3.8	0.0	0.1
feeders	Tussock	11.0		20.9	7.1	0.4	0.0
Filamentous	Inter-tussock	43.3		16.8	0.8	0.7	0.1
feeders	Tussock	8.2		3.0	2.3	0.2	0.0
Bacterial	Inter-tussock	9.7		2.0	3.8	0.9	0.2
feeders	Tussock	15.3		3.4	4.5	0.4	0.4
Miscellaneous	Inter-tussock	9.7		3.1	1.6	0.1	0.0
feeders	Tussock	4.8		3.0	1.5	0.1	0.1
Predators	Inter-tussock	0.0		0.0	0.1	0.2	0.0
Higher plant	Inter-tussock	70.8	62.5	80.1	35.1	1.9	
feeders	Tussock	31.5	70.6	72.2	28.1	3.1	
Filamentous	Inter-tussock	1.1	4.7	6.7	1.2	0.0	
feeders	Tussock	13.0	6.0	7.0	0.0	0.2	
Bacterial	Inter-tussock	8.5	20.1	2.4	1.2	0.3	
feeders	Tussock	11.2	6.0	2.8	4.2	0.2	
Miscellaneous	Inter-tussock	5.9	9.4	0.5	1.1	0.1	
feeders	Tussock	7.2	4.1	1.8	0.0	0.1	
Predators	Inter-tussock	0.3	0.8	0.0	0.5	0.1	
	Tussock	2.6	2.0	0.0	0.0	0.0	

not accurately determined, but rhizosphere samples from within the perimeter of F. novae-zelandiae plants showed relative stability over a few centimetres which approximates the 6cm diameter patches of high density indicated for enchytraeids in moorland soils (Peachey, 1963). The presence of single species in consecutive samples from the inter-tussock zone indicated that specific aggregates may occupy areas with a radius of at least 15cm. There was evidence of density gradients from the centres of greatest concentration.

No one factor has been isolated as having a greater or lesser effect in causing aggregation in such a complex system. The location of the dominant fescue tussocks had no apparent influence on the distribution pattern of nematodes in the inter-tussock zone and there was no obvious relationship between the plant species sampled and the composition of the nematode population. Havertz (1962) reported positive effects of plant species on the distribution of plant feeding nematodes and host specificity is a recognised phenomenon in phytopagous forms (Wallace, 1963). Similarly, plant species or varieties may induce localised modifications to the rhizosphere micro-flora (Buxton, 1957a, 1957b; Elkan, 1962; Rovira, 1965a)with possible effects on micro-herbivorous nematodes. But in view of the wide host ranges exhibited by species considered in Chapter 3, major effects of plants on the specific composition of nematodes in soil samples seem unlikely. However, different seasonal growth patterns of pasture species in a mixed sward could possibly stimulate localised nodes of high density.

The implications of contagious distributions are manyfold. Population estimates for faunistic surveys, population studies and economic nematology require data on the

nematode species present in certain areas of soil. It is apparent that large numbers of samples are required to obtain reliable estimates of populations or in the comparison of means in dynamics studies. This is emphasised by the fact the samples in the present investigation were taken from a small area which was selected for uniformity. The validity of many comparisons of numbers of particular nematode species between areas, or of annual variation of species must be questioned where small numbers of samples have been taken. The problems involved in estimating levels of infestation of nematodes in clumped distributions have been recognised by phytopathologists with regard to quarantine regulations for the potato-root eelworm Heterodera rostochiensis Woll., (e.g.Anscombe, 1950; Peters, 1951; Widdowson, 1962). Similar problems face future workers as nematodes become implicated in further plant disease syndromes. The importance of distribution studies on nematode populations from a wide range of habitats cannot be over emphasised from this point of view.

Pasture improvement significantly increased the density of the nematode population at Broken River. The increase was mainly due to an increase in plant feeding forms. Havertz (1962) reported a similar 'population explosion among the parasitic forms', when virgin soil was cultivated. Seinhorst (1966b)considered that at a particular density, of plant parasitic nematodes "available food is just sufficient to maintain the population" and this density he termed "equilibrium density". Raising the nutrient status of the soil to support a highly productive sward of exotic pasture species would increase the available food supply and thence result in a new equilibrium density. It has been suggested that increased rate of production of organic matter associated with greater environmental stability or predictability will be accompanied by a greater

species diversity (e.g.Connell and Orias, 1964; Slobodkin and Sanders, 1969). In the absence of invaders, the newly created niches must be filled over the short term by the existing species. With the notable exception of Criconemoides sp., which was absent from all but one sample from the cultivated soil, the dominant plant parasitic species are the same on both sites at Broken River. Similarly, there was little change in species composition for nematodes of other feeding groups. The effect of grassland improvement on numbers of nematodes in those groups was small relative to the effect on higher plant feeding forms. Previous workers reported little qualitative change for fungi (Thornton, 1960b), bacteria (Stout, 1960a), and protozoa (Stout, 1960b) between these soils, but noted an increase in the numbers of isolates from the cultivated soil. It seems that the increase in potential food is not necessarily available to nematodes; other environmental factors may apply which restrict a 'proportionate' increase in the number of nematodes.

6. POPULATION DYNAMICS

6.1 INTRODUCTION

Annual variation in nematode faunas was reported by early workers (e.g.Micoletzky, 1922; Seidenschwarz, 1923). More recent studies have confirmed that seasonal fluctuations in plant parasitic and free-living nematodes (e.g. Ferris and Bernard, 1961; Winslow, 1964; Zuckerman, Khera and Pierce, 1964; Banage, 1966; Szczygiel, 1966; Yuen, 1966; Prasad and Jha, 1970).

In general, soil nematodes exhibit high population levels with modest regular fluctuations (Dao, 1970). As the Broken River environment is characterised by marked seasonal extremes, it was considered that fluctuations of nematode numbers may be emphasised, thereby providing an opportunity to assess the importance of environmental factors associated with changes in the nematode fauna.

Interpretation of interactions between nematodes and their environment under field conditions is complicated by the heterogeneity of the soil. Environmental effects are complex and interdependent. However, by measuring changes in the nematode fauna and concurrent changes in basic environmental parameters, associated trends may be identified. With a knowledge of the biology of the main species comprising the fauna, and a knowledge of their distribution patterns, it was considered that a realistic evaluation of these associations might be achieved.

6.2 GENERAL METHOD

Following the reasoning developed in Chapter 5, two patterns of sampling were carried out between December, 1968, and February, 1970.

i) nematodes were extracted from a series of samples taken to a standard depth to obtain an estimate of variation in the horizontal plane.

ii) samples were taken at a series of levels in the soil horizon to estimate variation in the vertical plane.

For simplicity, environmental fluctuations were measured using three basic parameters; soil temperature; soil moisture; and loss on ignition (as an index of organic matter). Temperature, moisture and organic matter (as related to food influences) are dominant components of the soil environment. Although a multiplicity of other factors may be involved in complex interactions with them, it is suggested that the majority of environmental influences are derived from these effects.

In an attempt to identify specific food influences on the distribution and abundance of nematodes, the seasonal trends and distribution of bacteria and fungi were investigated in samples from the vertical plane.

6.3 VARIATION IN THE HORIZONTAL PLANE

6.3.A Method

6.3.A.I Sampling

On the basis of preliminary analyses (see Chapter 4) sample stations were confined to tussock growing in good condition on improved grassland. The sampling area of 60 x 40 metres was pegged out into 100 sub-plots of equal size, each identified by two boundary co-ordinates. The total area contained approximately 1,000 fescue tussock plants. Forty soil cores were taken through tussock plants on each of eight sampling intervals over a period of 14 months. Forty-eight to 58 days elapsed between samples. The location of sampling stations was identified by two series of numbers taken at random from a table of random

numbers. The first series of co-ordinates identified the sub-plot, and the second, the position of the tussock to be sampled within the sub-plot. Tussocks were located using a set of standard dividers; the nearest plant to the intersect of the co-ordinates was sampled.

The 40 cores which constituted each sample were placed in groups of four and trimmed to a length equivalent to a depth of 10cm. From each core in a group, 2.5cm units were removed from the top of the first, to the bottom of the fourth (Figure 46), and this soil was used for measurement of environmental parameters. The remainder, from which nematodes were extracted, was therefore the equivalent of four 7.5cm cores. During the dry summer months when soil tended to crumble, 25% of the volume of each sample was combined for testing and nematodes extracted from the rest.

6.3.A.II Extraction and processing

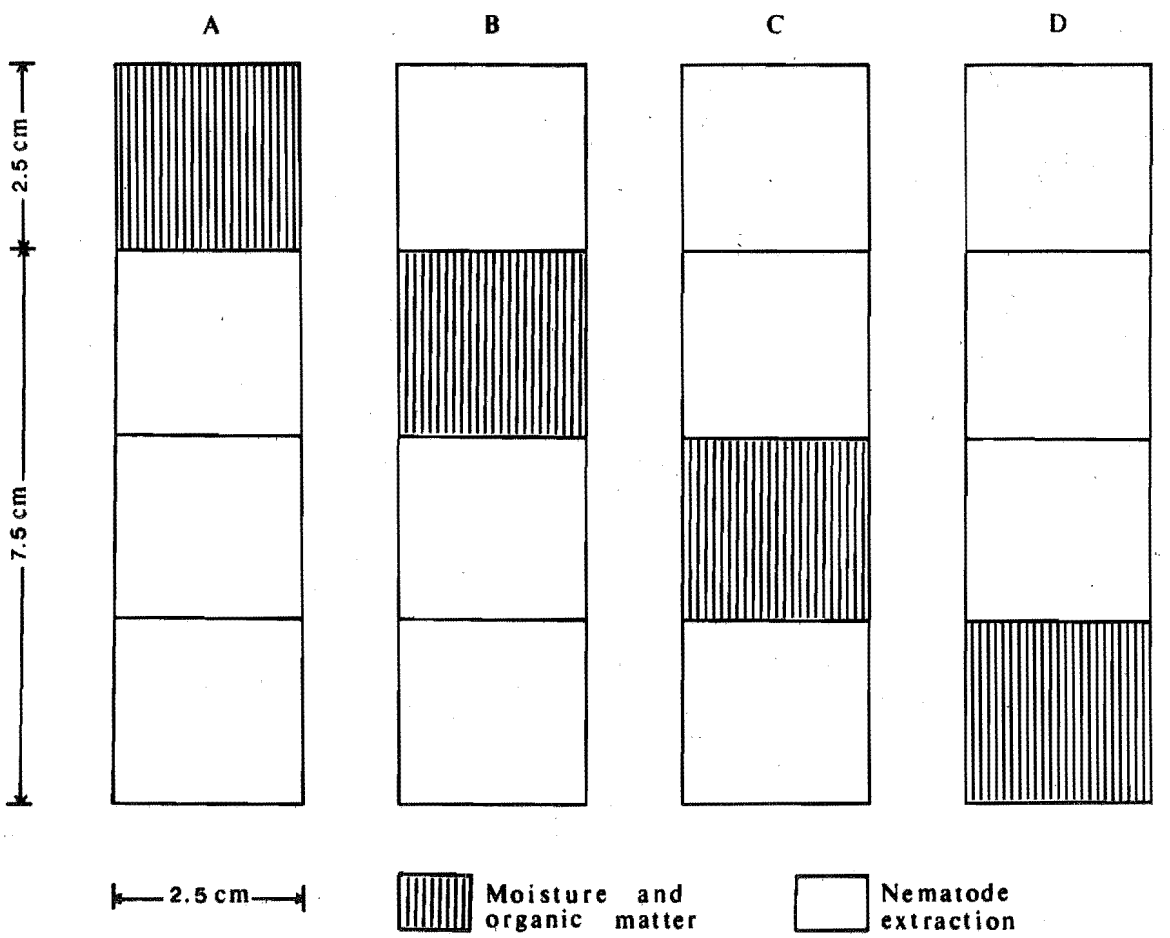
Each group of four soil cores was treated as a sample in subsequent processing. Following dispersion of the sample in about four litres of water, an aliquot consisting of 25% of the total volume was obtained by passing the suspension through the separating column described in Chapter 2.3C. Elutriation, concentration and counting proceedures were discussed in Chapter 2.

6.3.A.III Measurement of environmental parameters

Soil moisture was calculated for each sample as a percentage of the oven dry weight after drying approximately 10g of soil for 12 hours at 105-110^{o}C (Metson, 1956).

Loss on ignition was calculated as a percentage of the weight of about 10g of oven dry soil after ignition at 550^{o}C (Howard, 1964).

FIGURE 46. Division of soil cores before extraction of nematodes and soil testing.


A
B
C
D
2.5 cm
7.5 cm
2.5 cm
Moisture and organic matter
Nematode extraction

Temperature was recorded between 1pm and 3pm at each sampling, by inserting a mercury-in-glass thermometer to a depth of 5cm into the rhizosphere of 10 fescue tussock plants, from which a mean temperature was calculated. Ideally, continuous temperature recordings would be taken. To achieve this end, temperature integrators were constructed using thermistor probes, a mercury current integrator (Curtis meter) and a mercury battery (Duncan, pers. comm., 1968). Delays in obtaining components meant that the instruments were not completed until mid-way through the sampling programme. A bank of seven units was placed in position during June and gave reliable results for two sampling periods, but further readings could not be used. MacFayden and Wells (1968) described the integrator in detail. From their discussion, modifications could be made to correct the weaknesses.

6.3.A.IV Analysis of data

Transformation of data: Field data of an ecological nature often requires transformation to satisfy the assumptions of usual statistical tests (additivity, homogeneity of variances and normality). Taylor (1961) found that variance is related to the mean by a simple power law. It holds for a continuous series of distributions from regular through random to highly aggregated (Southwood, 1968), and is expressed by $s^2 = am^b$, where s^2 is the variance, m is the mean, and a and b are constants: 'a' is largely a sampling factor and b is an index of aggregation (Taylor, 1961). Taylor showed that as the variance varies with the mean in this way, the appropriate transformation function to stabilise the variance is of the form: $f(x) = \int Q\, m^{-b/2}\, dm$ where q is a constant.

The regression of mean on variance of data for total nematodes, ecological feeding groups (as defined in Chapter 4.2) and several common species was calculated. Where the

regression was shown by a 't' test to be significant (Duncan, pers. comm., 1971), that is, the variance is not independent of the mean, transformation using Taylor's exact exponent was carried out.

Regression analysis: Step-wise multiple regression analysis was used to investigate the significance of the relationships between variation of environmental factors and variation of the nematode fauna. Nematode numbers were assumed to be dependent on environmental factors. Product-moment correlation coefficients for each variable in the set with each other variable were computed. Additive covariation of environmental factors with nematode numbers was measured in multiple correlation coefficients. At each step, the variable entered into the regression equation is that which explains the greatest amount of variation between it and the dependent variable (that is, the variable with the highest partial correlation with the dependent variable).

6.3.B Results and discussion

6.3.B.I Variation with time

Variation of total nematodes, ecological feeding groups, and common species are summarised in Figures 47 and 48. Mean numbers per ml of soil with 95% confidence limits about the mean are plotted for each sampling period.

The graph of variation of the total nematode fauna with time shows a summer (February sample) maximum which is reflected in the curves for higher plant feeders and filamentous feeders. The peak appears earlier for bacterial feeders and is maintained later into the season for miscellaneous feeders. Bacterial feeding species frequently possess a short generation time and hence a more rapid response to environmental changes could be expected. On the other hand miscellaneous feeders are long-lived and the

FIGURE 47. Variation of total numbers of nematodes and numbers in ecological feeding groups in samples taken during a 14 month sampling programme. (95% confidence limits are shown)

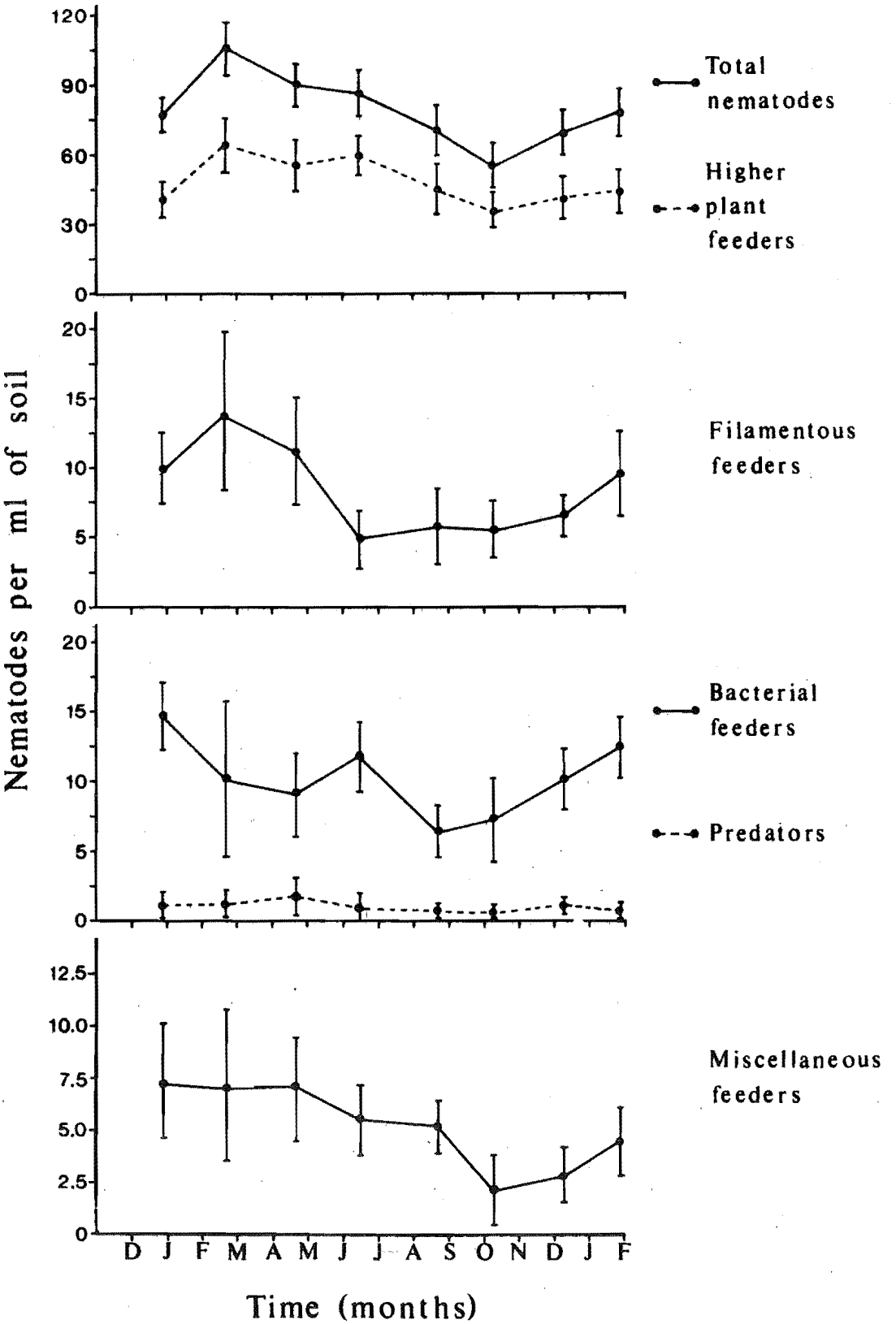
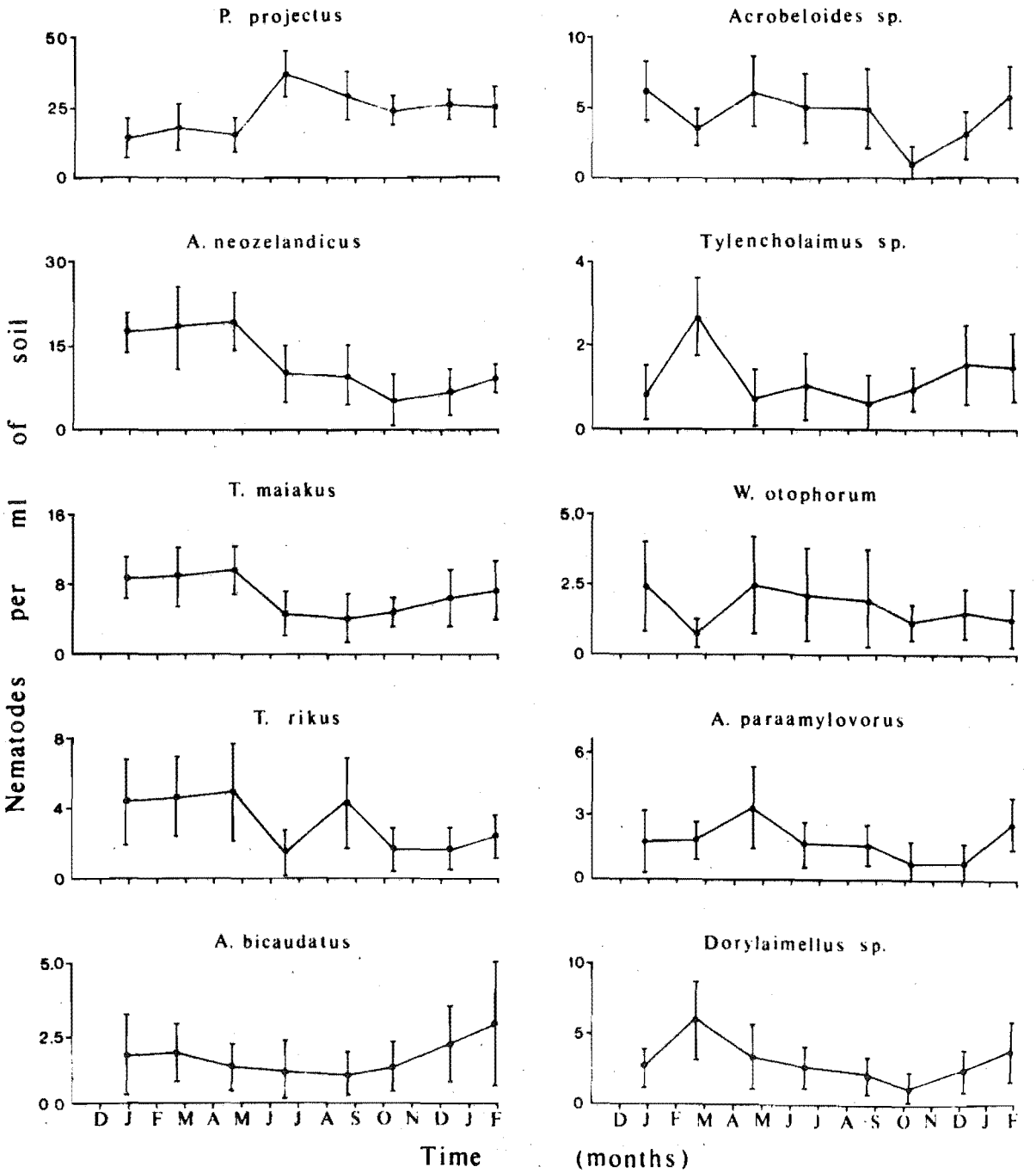

120
90
60
30
0
Total nematodes
Higher plant feeders
20
15
10
5
0
Filamentous feeders
20
15
10
5
0
Bacterial feeders
Predators
12.5
10.0
7.5
5.0
2.5
0
Miscellaneous feeders
Nematodes per ml of soil
D J F M A M J J A S O N D J F
Time (months)

FIGURE 48. Variation of populations of 10 species of nematodes in samples taken during a 14 month sampling programme.


P. projectus
50
25
0
Acrobeloides sp.
10
5
0
A. neozelandicus
30
15
0
Tylencholaimus sp.
4
2
0
T. maiakus
16
8
0
W. otophorum
5.0
2.5
0
T. rikus
8
4
0
A. paraamylovorus
6
3
0
A. bicaudatus
5.0
2.5
0 0
Dorylaimellus sp.
10
5
0
Nematodes per ml of soil
D J F M A M J J A S O N D J F
Time
D J F M A M J J A S O N D J F
(months)

recorded effect of environmental change on numbers of nematodes can be expected to be delayed as indicated by the curve. A similar trend during the summer-autumn period is apparent for higher plant feeders, but this is considered to be related to a particular feature of the biology of the most common species of the higher plant fauna (see discussion below).

Despite the effects of aggregation and sampling error evident in the 95% confidence limits about the mean, the graphs for each species (Figure 48) show general trends toward winter minima and summer maxima. For the higher plant feeding species A. neozelandicus and T. maiakus, a summer-autumn peak is apparent, with a decline in numbers during the winter period but decreasing with the onset of summer conditions. However, P. projectus exhibits a peak during winter which is maintained with only a slight decrease into spring. Rhoades and Lindford (1961a) have shown that fourth stage juveniles of P. projectus are able to survive for long periods in a non-feeding condition. With the onset of winter, an increase in fourth stage juveniles could be expected and indeed was evidenced by the large proportion of this stage extracted from samples. It is suggested that the increase in P. projectus resulted from the survival of juveniles in this form and possibly from an increase in the efficiency of extraction as the size of individuals comprising the population increased. (see Chapter 2.5). The apparent increase in numbers during winter contributes largely to the gradual decline of the total higher plant feeding fauna during winter.

6.3.B.II Variation with environmental factors

Transformation of data in the step-wise multiple regression programme was carried out using Taylor's exact transformation according to the significance of the

regression of variance on mean (Table 39).

The significance of the relationships between changes in nematode numbers and changes of environmental factors is shown in Table 40. Product-moment correlation coefficients in columns one to three express the significance of the association of each environmental factor with each level of the fauna considered. The factor with the highest order of association when the remaining variables were held constant is entered in column four. Column five shows the additive effects of two environmental factors, and column six, the effect of three environmental factors. The order of entry of independent variables is indicated in columns seven to nine.

Correlations between the occurrence of nematodes and fluctuations in the environment are low (Table 40). Significant associations are evident only for filamentous feeders, P. projectus, T. maiakus, and Tylencholaimus sp. No one factor is consistently 'associated' with changes in numbers. However, in the analysis, the assumption is made that cause and effect are synchronous. Further, measurement of fluctuations is based on a chronological time scale without regard to the tolerance of nematodes to environmental changes or to physiological time. In a biological system existing in a buffered environment, such assumptions may be invalid.

Lag effects in the present data are demonstrated by plotting total nematode numbers at each sampling interval against each environmental factor (Figure 49). The opposite rotation of the ellipses described in the regression of numbers on temperature and on soil moisture is consistent with the negative correlation between soil temperature and soil moisture (Table 41).

TABLE 39: Determination of transformation exponents using Taylor's power law.

Category	Regression of variance on mean		Significance of regression by 't' test	Exponent
	Constant	Gradient		
Total nematodes	1.6776	0.3279	0.6472	0.83605
Higher plant feeders	0.2235	1.1838	1.6924	0.40809
Filamentous feeders	-0.6178	1.9942	3.3881**	0.00289
Bacterial feeders	0.6595	0.5425	0.5333	0.72877
Miscellaneous feeders	0.3491	0.8542	1.5884	0.57291
Predators	0.0684	1.5732	3.0797*	0.21338
P. projectus	0.7374	0.8970	1.2393	0.55149
A. neozelandicus	1.0701	0.5452	0.8866	0.72740
T. maiakus	0.6967	0.6171	1.1878	0.69147
T. rikus	0.1154	1.6367	6.6373**	0.18164
A. bicaudatus	0.0384	2.0489	6.3996**	-0.02446
Tylencholaimus sp.	-0.0012	0.8166	2.9419*	0.59171
Acrobeloides sp.	0.1704	1.0517	4.0634**	0.47413
W. otophorum	-0.0281	2.0879	4.9808**	-0.04397
A. paraamylovorus	0.1813	0.5394	1.9902	0.73030
Dorylaimellus sp.	0.1354	1.2863	6.5953**	0.35686

t(6 df)
0.05 = 2.447*
0.01 = 3.707**

TABLE 40: Significance of the association between fluctuations in nematode numbers and fluctuations of soil moisture (M), soil organic matter (O), and soil temperature (T)

Nematode category	Product-moment correlation coefficients			Multiple correlation coefficients			Order of entry
	M	O	T	1	2	3	
Total nematodes	0.0522	-0.0394	0.0663	0.066	0.181	0.190	T M O
Higher plant feeders	0.2164	0.0318	-0.2310	0.231	0.248	0.254	T M O
Filamentous feeders	-0.2054	-0.1804	0.4106**	0.411**	0.419	0.428	T M O
Bacterial feeders	-0.1264	-0.1068	0.2300	0.230	0.234	0.238	T M O
Miscellaneous feeders	-0.0807	-0.0514	0.0279	0.081	0.099	0.102	M T O
Predators	-0.0890	-0.0212	0.0606	0.089	0.090	0.090	M O T
P. projectus	0.2292	0.2349*	-0.4325**	0.433**	0.468	0.489	T M O
A. neozelandicus	-0.0944	-0.1636	0.1349	0.164	0.184	0.190	O T M
T. maiakus	-0.1294	-0.1915	0.2989**	0.299	0.344*	0.371	T M O
T. rikus	-0.0420	-0.0746	0.1521	0.152	0.176	0.182	T M O
A. bicaudatus	0.0910	0.0382	-0.0549	0.091	0.091	0.092	T O M
Tylencholaimus sp.	-0.2756*	0.3533**	0.1836	0.353*	0.396	0.397	O T M
Acrobeloides sp.	-0.0684	0.1581	0.0187	0.158	0.161	0.169	O T M
W. otophorum	0.0698	0.1174	-0.0244	0.115	0.120	0.126	O M T
A. paraamylovorus	0.0700	-0.0918	0.0212	0.092	0.144	0.186	O M T
Dorylaimellus sp.	-0.0641	-0.1135	0.1180	0.118	0.141	0.148	T O M

r (70df)
0.05 = 0.232*
0.01 = 0.302**

R (70df)
0.05 = 0.324*
0.01 = 0.386**

FIGURE 49. Relationship between variation of total nematodes per ml of soil and variation of soil temperature, soil moisture, and loss on ignition. Order of sampling indicated by numbers at each data point.

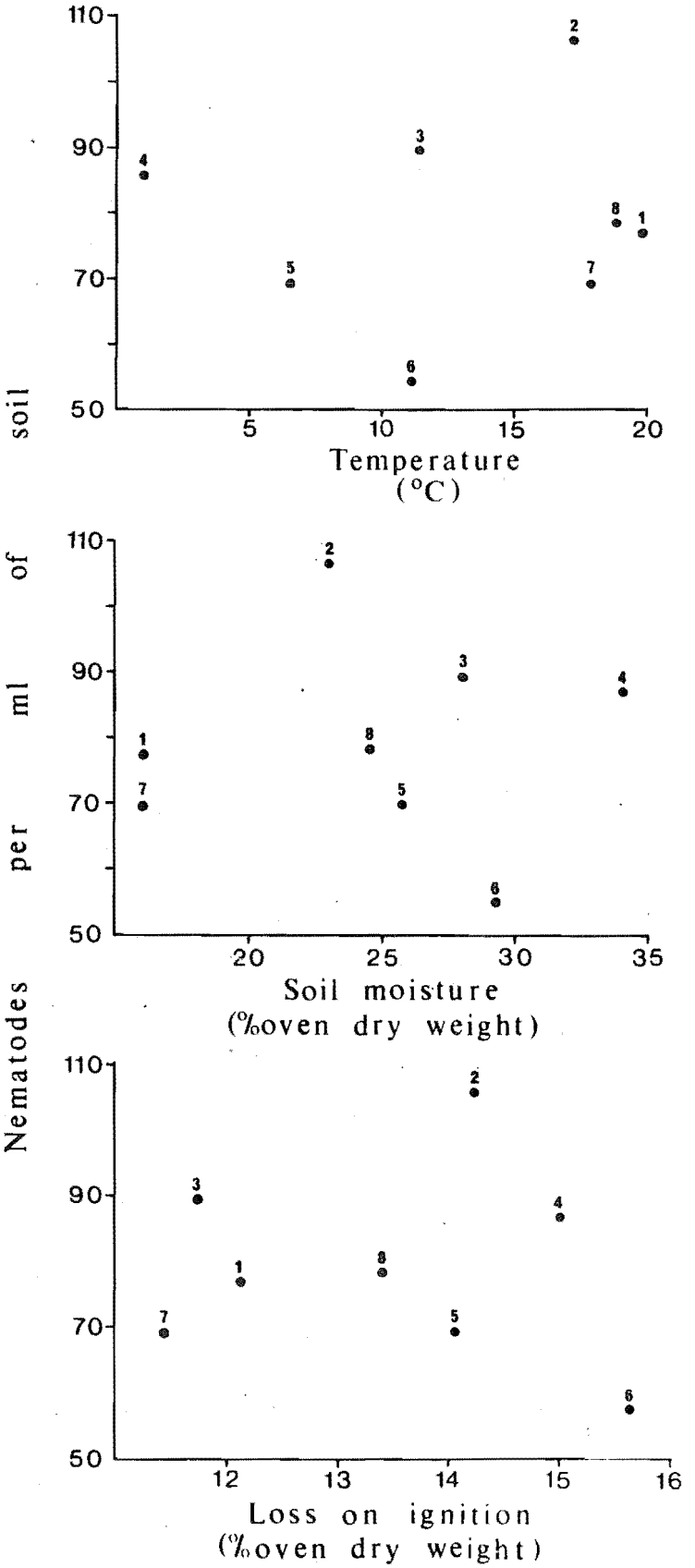

Nematodes per ml of soil
110
90
70
50
5
10
15
20
Temperature (°C)
20
25
30
35
Soil moisture (%oven dry weight)
12
13
14
15
16
Loss on ignition (%oven dry weight)

TABLE 41: Significance of the association between seasonal fluctuations of soil moisture, loss on ignition and soil temperature.

	Product-moment correlation coefficients		
	Soil moisture	Loss on ignition	Soil temperature
Soil moisture	1.0000	0.2257	-0.4818**
Loss on ignition	0.2557*	1.0000	-0.2299
Soil temperature	-0.4818**	-0.2299	1.0000

r (70df)
0.05 = 0.232*
0.01 = 0.302**

The relationship of organic matter is more obscure. As the formation and decomposition of organic matter is dependent on the activities of the soil flora and fauna, which in turn are limited by soil temperature and soil moisture, complicated interactions are involved, some of which are considered in subsequent discussion.

6.3.B.III Interpretation of the lag phase

The discrepancy between the recorded environment at the time of sampling and the actual environment preceding sampling must account for part of the lag phase. As the recorded environment is not a measure of the 'mean' environment for the period between samples, a minimum lag of a few days to a maximum lag of slightly less than a sampling interval could be expected. The direction of rotation of the theoretical ellipses (indicated by sequential sample numbers) shown by regression of nematode numbers on soil temperature

and soil moisture at various levels of the fauna (Figures 50 and 51), indicate that temperature effects contribute largely to the hysteresis. Clearly, the change in temperature precedes changes in nematode numbers by a considerable period, whereas the general trend of soil moisture is preceded by fluctuations in nematode numbers.

The approximate interval between temperature change and nematode response was calculated from the relationship; $A = \frac{B \times \theta}{360^{o}}$ (Robson pers. comm., 1971) where A is the lag period, B is the total sampling period of 395 days, and $\theta = \text{Sin}^{-1} \frac{X1}{X2}$ in degrees calculated from the plotted ellipse (see Figure 52). Calculation of the mean lag period of 76 days between the total nematode fauna and temperature is shown in Figure 52. Similar calculations for ecological feeding groups indicate a lag phase of 69 days for higher plant feeders, 61 days for filamentous feeders, 55 days for bacterial feeders, 79 days for miscellaneous feeders and 76 days for predators. These intervals are merely approximations as they are based on only one environmental factor; they ignore the possibility that other factors may be limiting at particular times of the year, and further, the lag is assumed to be constant throughout the year. However, they serve to demonstrate that the 'average' lag period is greater than the interval between samplings. Hence it is concluded that the 'biological' tolerance of nematodes to environmental fluctuations, either in the short term or the long term, is implicated in the hysteresis effects.

Environmental changes may affect the rate of increase or decrease of a population, but because of the longevity of the species composing the fauna (see Chapter 4) a delay in the response is evident. For example, during the autumn-

FIGURE 50. Relationship between variation of numbers of nematodes in ecological feeding groups and species, with variation of temperature. Orders of sampling indicated by numbers at each data point.

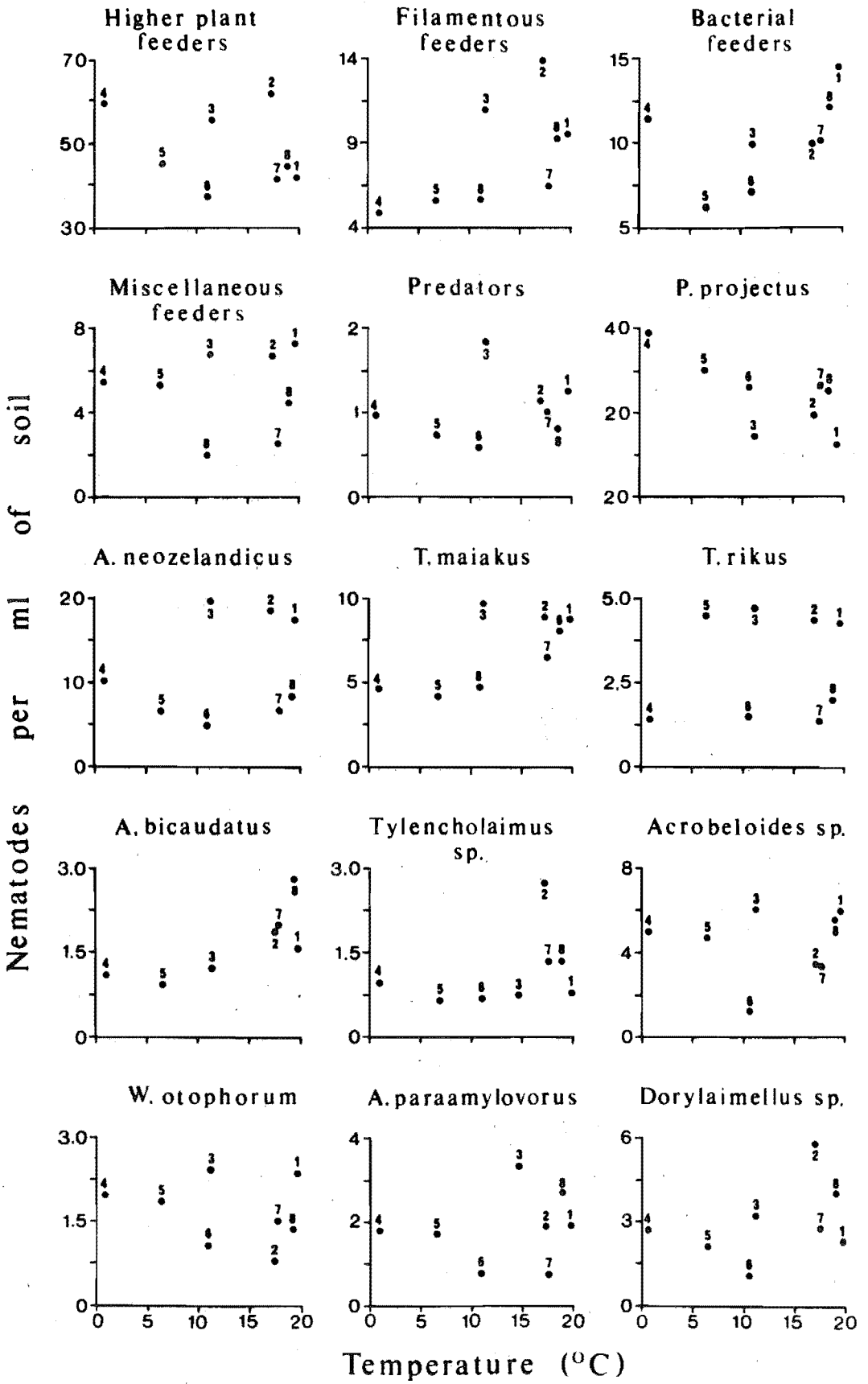

Higher plant feeders
Filamentous feeders
Bacterial feeders
Miscellaneous feeders
Predators
P. projectus
A. neozelandicus
T. maiakus
T. rikus
A. bicaudatus
Tylencholaimus sp.
Acrobeloides sp.
W. otophorum
A. paraamylovorus
Dorylaimellus sp.
Nematodes per ml of soil
Temperature (°C)

FIGURE 51. Relationship between variation of numbers of nematodes in ecological feeding groups, and species with variation of soil moisture. Order of sampling indicated by numbers at each data point.

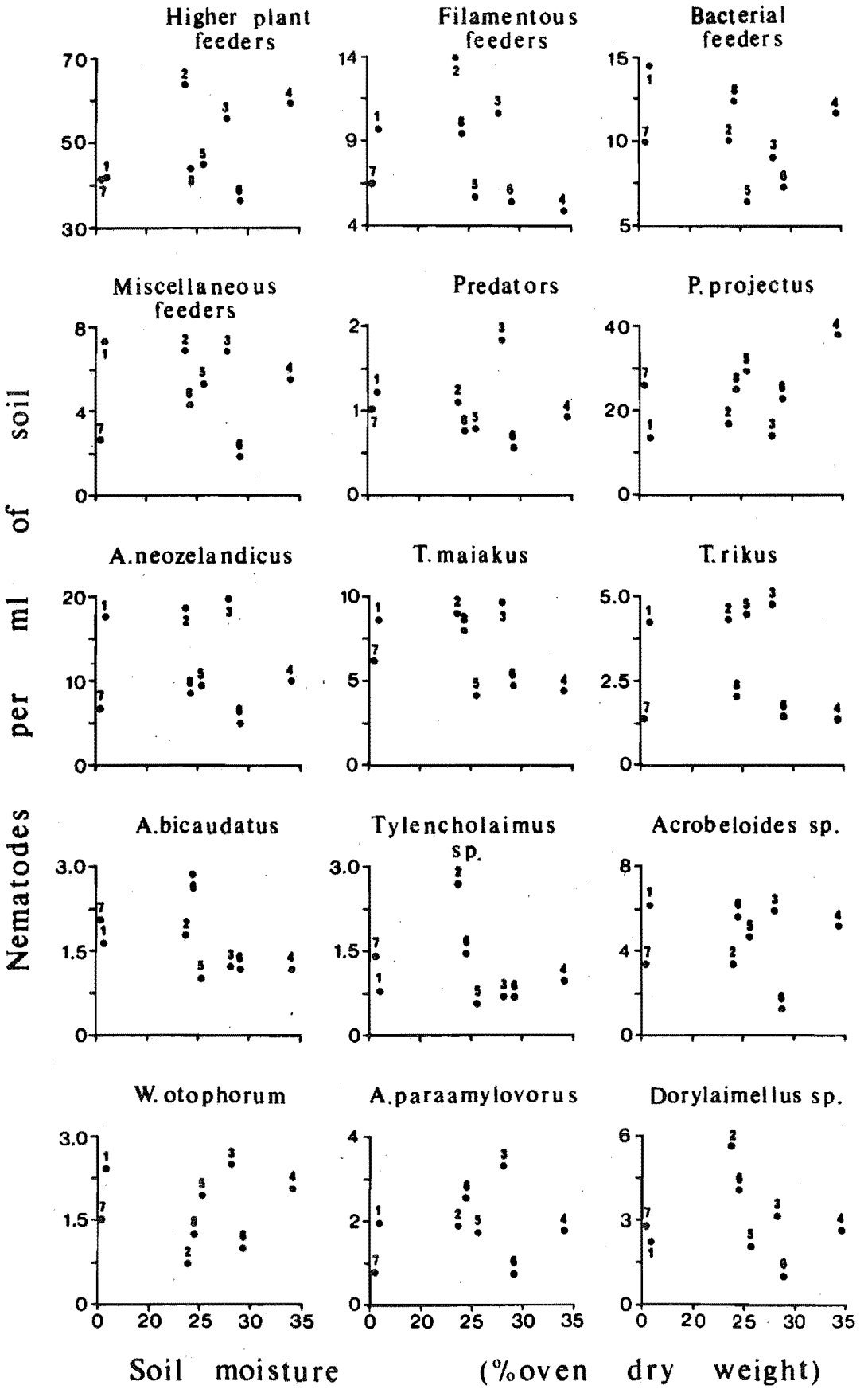
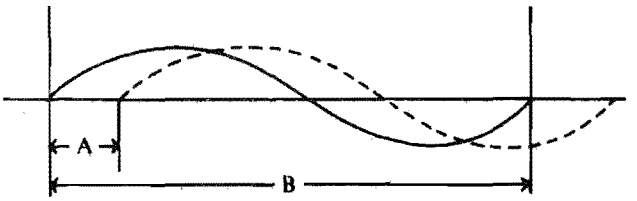
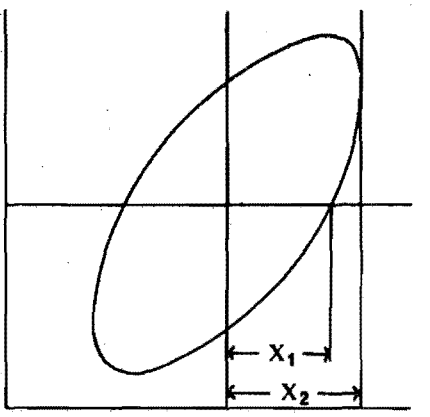

Higher plant feeders
Filamentous feeders
Bacterial feeders
Miscellaneous feeders
Predators
P. projectus
A. neozelandicus
T. maiakus
T. rikus
A. bicaudatus
Tylencholaimus sp.
Acrobeloides sp.
W. otophorum
A. paraamylovorus
Dorylaimellus sp.
Nematodes per ml of soil
Soil moisture (%oven dry weight)

FIGURE 52. Method of calculation of the lag phase between temperature fluctuations and nematode response.



$$A = \frac{B \times \Theta}{360}$$

$$\Theta = \arcsin\left(\frac{X_1}{X_2}\right)$$

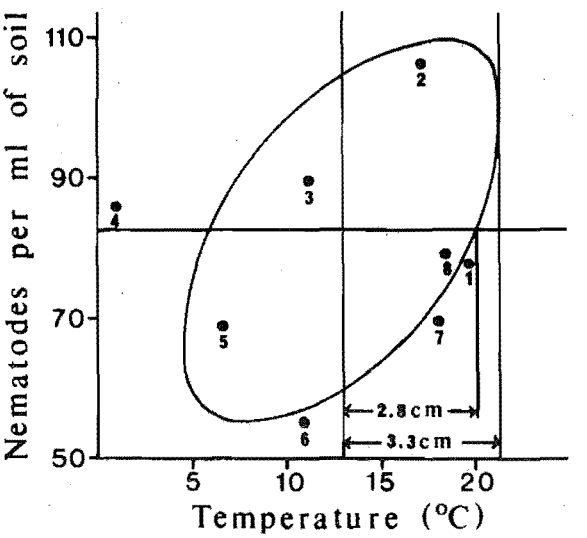


'Average' lag phase

for total nematodes

$$A = \frac{395 \times 69.3}{360}$$

$$= 76.03$$



winter period reproduction of a species may be limited by a decrease in temperature. However, until such time as the temperature reaches the lower cardinal limit for survival, or mortality from starvation or age occurs, a reduction in numbers would not be recorded. Because nematodes are poikilothermic, the response may be further delayed by the fact that a reduction in temperature below the optimum range for activity results in a reduction of metabolic activity which may be expressed as an increase in longevity on a chronological time scale. Conversely, as temperature increases during the spring-summer period and reproduction occurs, a lag between the onset of egg laying and the detection of a population increase can be expected.

The non-symmetrical shapes of the curves described in Figure 50, indicates that the lag is not of constant duration throughout the year. Much of the deviation from symmetry reflects sampling error, but the consistent displacement of sample 4 (June sampling) in the regression of total nematodes (Figure 49) and ecological feeding groups (Figure 50), suggests that the lag is of greatest duration during late autumn to winter. That is, during the period of decreasing temperature.

6.3.B.IV Effect of compensation for the lag phase

It follows from the previous section, that the identification of associations between fluctuations of environmental factors and fluctuations of nematode numbers, some adjustment for the lag interval must be made. The number of sampling occasions in the present series was not sufficient to allow the calculation of the specific lag intervals. However, the lag phase was shown to fall between one and two sampling periods. Thus further analyses were carried out in which adjustment for the lag phase was made by shifting nematode numbers back one and two sampling periods relative to environmental data. The effect of this adjustment on the

coincidence of environmental factors and nematode numbers is illustrated in Figure 53.

Comparison of the results presented in Tables 42 and 43 with results in which no adjustment was made (Table 40), demonstrates the significance of the lag phase. The adjustment in time has increased the significance of the relationship between changes in temperature and soil moisture and changes in nematode numbers. The consistent positive sign of the association with temperature indicates that this factor exhibits the most continuous limiting effect on nematode numbers throughout the year. Significant correlations between temperature and total nematodes, higher plant feeders and miscellaneous feeders are consistent where adjustment for one and two sampling intervals was made. A significant association with filamentous feeders and bacterial feeders is apparent for data analysed without adjustment, and after adjustment for one sampling period. High correlations were not recorded where nematode numbers were very low, either at the ecological feeding group level, or the species level (e.g. predators, T. rikus, A. bicaudatus, W. otophorum). The additive effects of soil moisture and organic matter shown by the increase in magnitude of the multiple correlation coefficients at some levels of the fauna indicate that at these levels, soil moisture and organic matter are involved in interactions with temperature and may be limiting at particular times of the year.

The results are discussed further considering each environmental factor separately.

Temperature: Wallace (1963) divided the influence of temperature on plant nematodes into five arbitrary phases: non-lethal low temperatures at which activity is inhibited; optimum temperatures; non-lethal high temperatures at which activity is inhibited; lethal low temperatures, and lethal

FIGURE 53. The effect of adjustment of nematode numbers relative to time of sampling and environmental factors recorded at sampling.
A) No adjustment. B) Adjustment for one sampling interval. C) Adjustment for two sampling intervals.

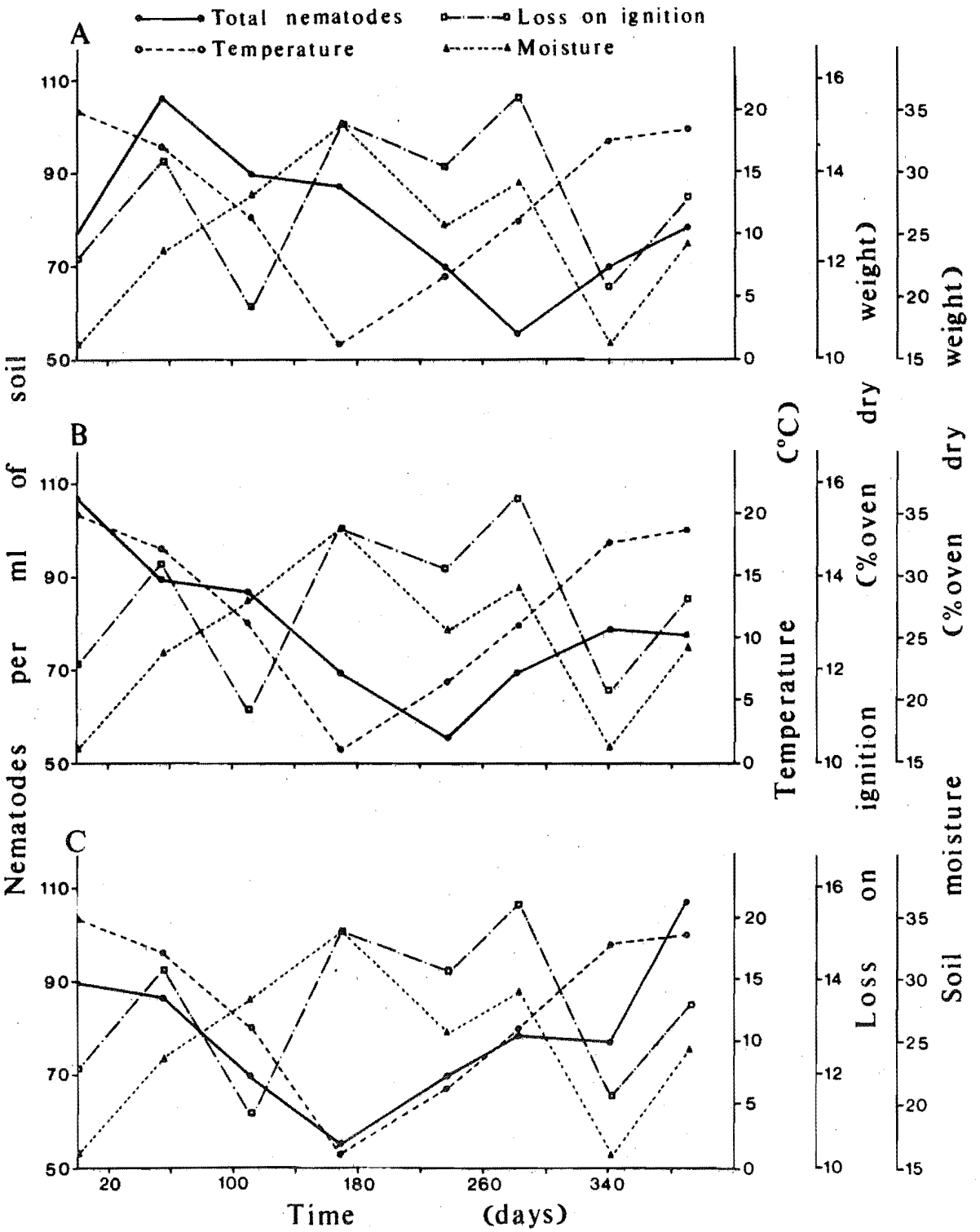

Total nematodes
Loss on ignition
Temperature
Moisture
A
B
C
Nematodes per ml of soil
Temperature (°C)
Loss on ignition (% oven dry weight)
Soil moisture (% oven dry weight)
Time (days)
110
90
70
50
20
15
10
5
0
16
14
12
10
35
30
25
20
15
20
100
180
260
340

TABLE 42: Significance of the association between fluctuations of nematode numbers and fluctuations of soil moisture (M), soil organic matter (O), and soil temperature (T), adjusted for one sampling interval.

Nematode category	Product-moment correlation coefficients			Multiple correlation coefficients			Order of entry
	M	O	T	1	2	3	
Total nematodes	-0.3758**	-0.2530*	0.4973**	0.497**	0.506	0.508	T O M
Higher plant feeders	-0.1833	-0.1562	0.2341*	0.234	0.248	0.249	T O M
Filamentous feeders	-0.3547**	-0.1435	0.4395**	0.440**	0.440	0.440	T M O
Bacterial feeders	-0.1500	-0.1619	0.3764**	0.376*	0.405	0.411	T M O
Miscellaneous feeders	-0.1267	-0.1688	0.2686*	0.269	0.301	0.323	T M O
Predators	0.0566	0.1140	0.0038	0.114	0.121	0.135	O T M
P. projectus	0.2572*	-0.0932	0.3645**	0.365*	0.430	0.430	T O M
A. neozelandicus	-0.2207	0.0393	0.3984**	0.398	0.440	0.454	T M O
T. maiakus	-0.3226**	-0.0124	0.3961**	0.396*	0.415	0.417	T O M
T. rikus	-0.0541	-0.0444	0.0992	0.099	0.107	0.109	T M O
A. bicaudatus	-0.0137	-0.1792	-0.0113	0.179	0.195	0.197	O M T
Tylencholaimus sp.	-0.1382	-0.2109	0.2464*	0.246	0.327	0.332	T O M
Acrobeloides sp.	-0.0759	0.0776	0.1567	0.157	0.206	0.209	T O M
W. otophorum	-0.1377	-0.0915	0.0855	0.138	0.146	0.147	M T O
A.paraamylovorus	-0.1652	-0.1069	0.2294*	0.229	0.232	0.235	T O M
Dorylaimellus sp.	-0.2458*	-0.1272	0.2351**	0.246	0.260	0.261	M T O

r(70df)
0.05 = 0.232*
0.01 = 0.302**

R(70df)
0.05 = 0.324*
0.01 = 0.386**

TABLE 43: Significance of the association between fluctuations of nematode numbers and fluctuations of soil moisture (M), soil organic matter (O) and soil temperature (T), adjusted for two sampling intervals.

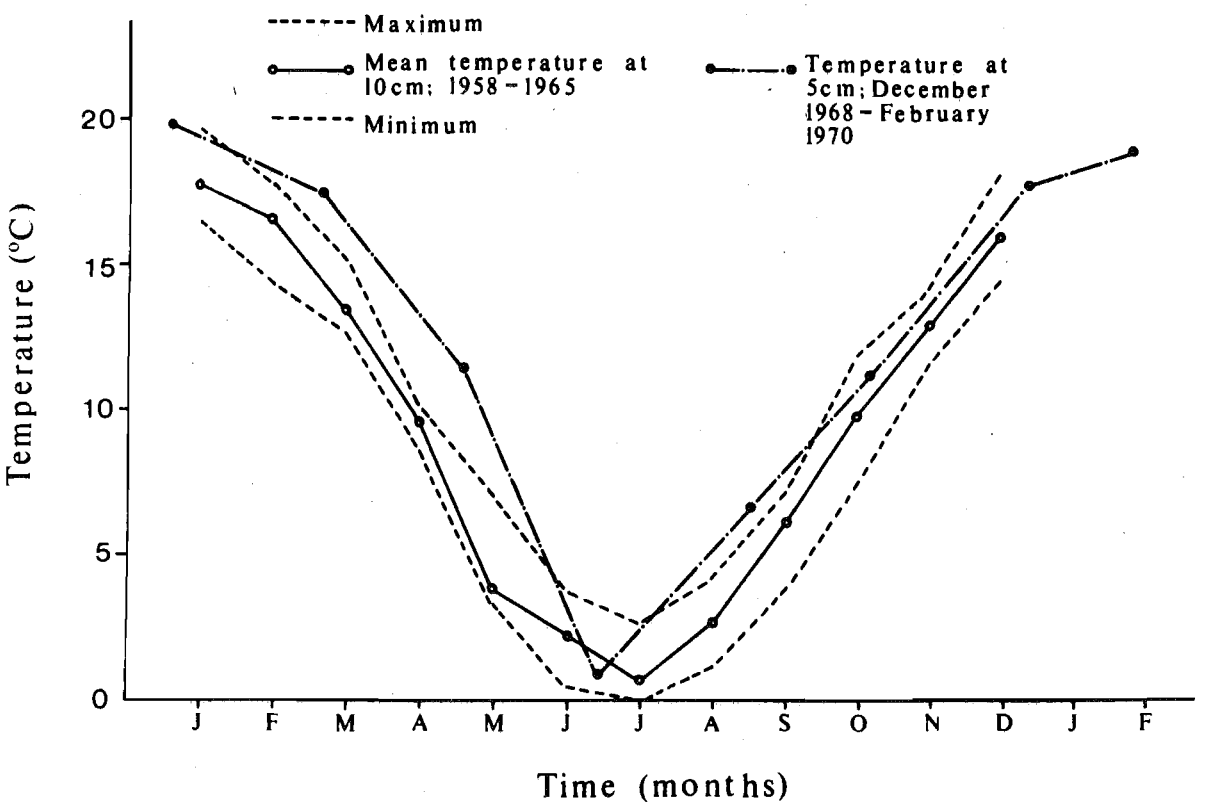
Nematode category	Product-moment correlation coefficients			Multiple correlation coefficients			Order of entry
Total nematodes	-0.4034**	-0.0756	0.6205**	0.620**	0.636	0.645	T O M
Higher plant feeders	-0.2114	-0.0591	0.4337**	0.434**	0.482	0.484	T M O
Filamentous feeders	-0.2902*	-0.0168	0.2441*	0.290	0.302	0.313	M O T
Bacterial feeders	-0.2757	0.1045	0.2278	0.276	0.351*	0.361	M O T
Miscellaneous feeders	-0.3871**	-0.2151	0.4668**	0.467**	0.471	0.471	T O M
Predators	-0.1171	-0.0878	0.0825	0.117	0.127	0.127	M O T
P. projectus	0.2877*	0.2294	-0.1778	0.288	0.315	0.326*	M O T
A. neozelandicus	-0.4978**	-0.1291	0.5220**	0.522**	0.541	0.547	T M O
T. maiakus	-0.2754*	-0.2062	0.2612*	0.275	0.297	0.304	M O T
T. rikus	-0.0156	-0.1734	0.0745	0.173	0.180	0.194	O M T
A. bicaudatus	0.1007	0.1163	0.1166	0.117	0.145	0.146	T O M
Tylencholaimus sp.	0.1246	0.1097	0.0534	0.125	0.216	0.338*	M O T
Acrobeloides sp.	-0.3189*	-0.1608	0.4014**	0.401**	0.403	0.404	T O M
W. otophorum	-0.0073	0.0873	0.1466	0.147	0.200	0.220	T O M
A. paraamylovorus	-0.2493*	-0.1027	0.3008*	0.301	0.302	0.302	T M O
Dorylaimellus sp.	-0.0655	0.0009	0.2242	0.224	0.263	0.270	T M O

r (70df)
0.05 = 0.232*
0.01 = 0.302**

R (70df)
0.05 = 0.324*
0.01 = 0.386**

high temperatures. From the literature reviewed by Wallace, (1963) and Dao (1970) it is apparent that the minimum and optimum temperatures for some activities differ between species. Wallace (1963) suggests that the low temperature range over which most phytoparasitic nematodes become inactive is about 5-15°C, the optimum range about 15-30°C, and the high temperature range for inactivity is 30-40°C. Dao (1970) considered that soil and plant nematodes are normally active and thriving at temperatures between 15° and 30°C and that nematodes become motionless in the ranges of 5-15°C and 30-40°C. Soil temperatures recorded at Broken River did not exceed 20°C (Figure 54). Hence it seems unlikely that nematode activity would be limited by high temperature at any time during the summer season. Egg development for many of the common species at Broken River was reduced at 10°C and did not occur at 5°C (see Chapter 4.12), which is generally consistent with the records reviewed by Wallace (1963) and Dao (1970). Significant population increases are therefore improbable while soil temperatures remain below 10°C. Comparison of the temperature records from the present study with average monthly temperatures at a depth of 10cm from Department of Agriculture records taken at the same site for the period 1958 to 1965 show that the soil is below this level for about six months of the year (Figure 54). Nematode mortality due to the lethal effects of temperature, starvation, parasitism, age, or to inhibitory effects of other environmental factors would occur without replacement during this period. Many nematode species are able to survive temperatures below 5°C in a quiescent or anabiotic state (Wallace, 1963; Dao, 1970). But Wallace notes that active stages are probably killed at or below freezing point, depending on the duration of exposure. Soil temperatures below 5°C occur between May-June to August September at Broken River

FIGURE 54. Variation in soil temperatures recorded at Broken River. Records for 1958-1965 obtained from Mr A. R. Dingwall of the Department of Agriculture.


Maximum
Mean temperature at 10cm; 1958 - 1965
Minimum
Temperature at 5cm; December 1968 - February 1970
Temperature (°C)
20
15
10
5
0
J
F
M
A
M
J
J
A
S
O
N
D
J
F
Time (months)

(Figure 54); freezing of the top few centimetres of soil frequently occurring during June and July. The combination of prolonged cold conditions with intermittent freezing appears to be the primary factor involved in the decline of nematode numbers during winter.

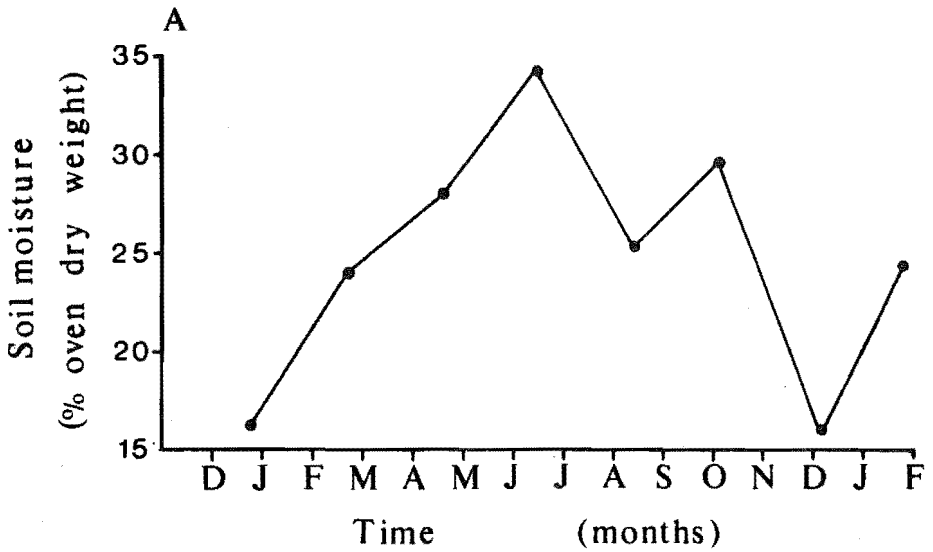
Soil moisture: Several workers have shown that survival, activity, or population growth of nematodes is limited by wet or dry conditions (e.g. Godfrey, 1926; Wallace, 1954, 1955a, 1955b, 1956a, 1956b, 1960; Peacock, 1957; Johnson, 1958; McGlohon, Sasser and Sherwood, 1962; Radewald and Takeshita, 1964; Kable and Mai, 1968; Wyss, 1970). Activity of nematodes is confined to the lower part of the pF scale; from pF 0 to some point between pF 2.7 and 4.0 (Nielsen, 1967). However, the percentage of water held at a given moisture tension varies with soil type (Buckman and Brady, 1960) and the moisture tension necessary for satisfactory population growth may also vary with soil type (Kable and Mai, 1968). Total suction potential for samples of soil from Broken River of known moisture content were determined using thermocouple psychrometer (see Chapter 7.2). Comparison of the resultant curve with the soil moisture data for the sampling period (Figure 55) shows that for most of the year the suction tension ranged from about 1 to 10 bars (pF 3-4).

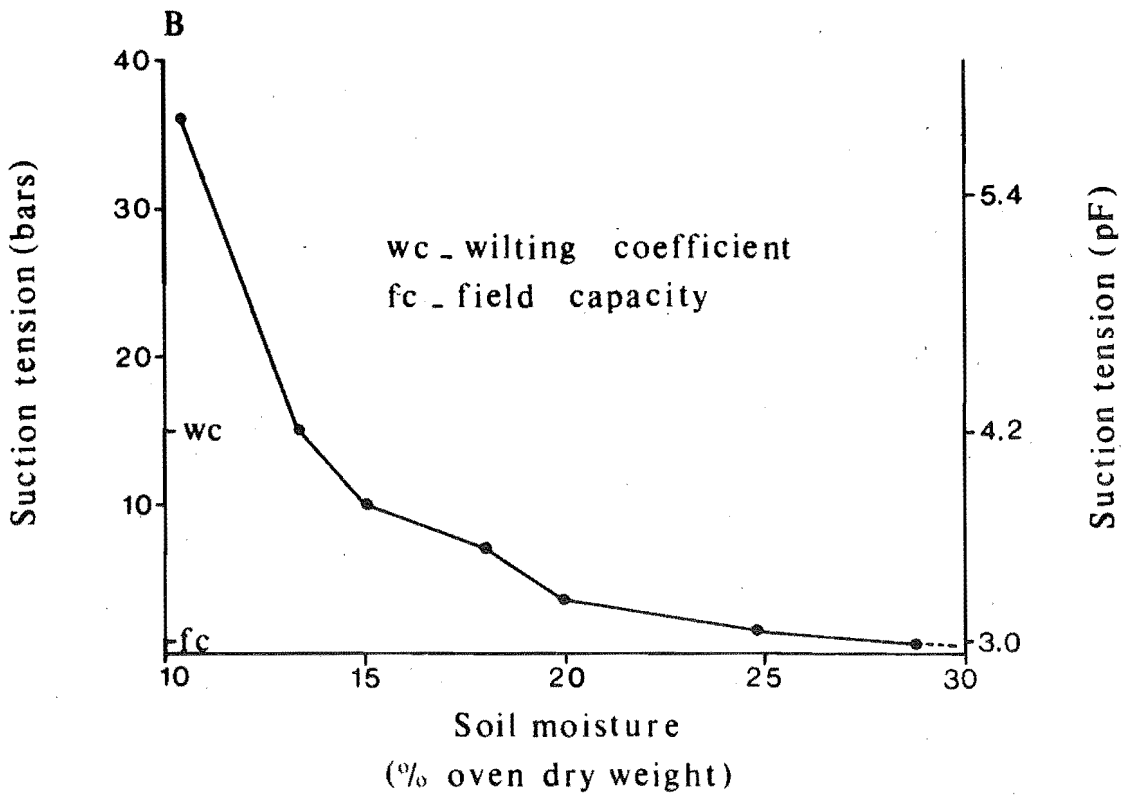
Wallace (1960) reported that larval mobility of Heterodera rostochiensis in sand and clay was greatest at moisture tensions slightly less than 0.1 bars (pF2), but that high mobility occurred in peat at moisture contents less than field capacity ($<$0.3 bars; $<$ pF 2.5). Reproduction of Hemicycliophora arenaria in a sandy loam under moisture regimes of less than 10 centibars ($<$ 0.1 bars) and greater than 50 centibars ($>$ 0.5bars) was 40% less under conditions of high soil moisture at 15^{o}C and 46.6%

less at 30°C (Van Gundy, McElroy, Cooper and Stolzy, 1967). Kable and Mai (1968) suggested that the rate of population increase of Pratylenchus penetrans is greatest at moderate soil moisture tensions (pF 2-3; 0.1-1.0 bars), and is least at very low or very high moisture tensions: they found that the higher the silt and clay content of the soil, the greater is the moisture tension necessary for satisfactory population growth. The Broken River soil is classified as a dark brown fine sandy loam (Chapter 2.2.D). From the literature, it would seem that excess soil moisture is unlikely to become inhibitory until it exceeds field capacity (0.3 bars; pF 2.5). Moisture tensions exceeding field capacity possibly occurred during mid-winter (Figure 55), but at this time temperatures were less than 5°C. High soil moisture has been associated with low oxygen availability (Van Gundy et al, 1967). But respiration is reduced at low temperatures and, in many species of nematodes it is not detectable at 5°C (Bhatt and Rohde, 1970). Kable and Mai (1968) reported that survival of P. penetrans was high in saturated soils at 0-6°C but decreased with increasing temperature up to 30°C. Hence with the reduction in metabolic activity and oxygen requirement associated with low temperatures at the time soil moisture is high at Broken River, it is suggested that excess moisture is unlikely to be major limiting factor on nematode population growth.

Maximum soil moisture suctions of about 10 bars (pF 4.0) were recorded during summer. The tolerance of nematodes to desiccation varies considerably between species (Wallace, 1963; Van Gundy, 1965; Nielsen, 1967). For example, Wyss (1970) showed that the tolerance of P. penetrans, Rotylenchus robustus (de Man, 1876), Filipjev, 1936, Trichodorus pachydermus Seinhorst, 1954, and Trichodorus similis Seinhorst, to desiccation decreased from about pF 3.7, whereas Tylenchorhynchus dubius (Bütschli, 1873) Filipjev, 1936

FIGURE 55. Variation in soil moisture recorded at Broken River. A) Moisture curve. B) Relationship between soil moisture (% oven dry weight) and suction tension (bars).


A
Soil moisture
(% oven dry weight)
35
30
25
20
15
D J F M A M J J A S O N D J F
Time (months)

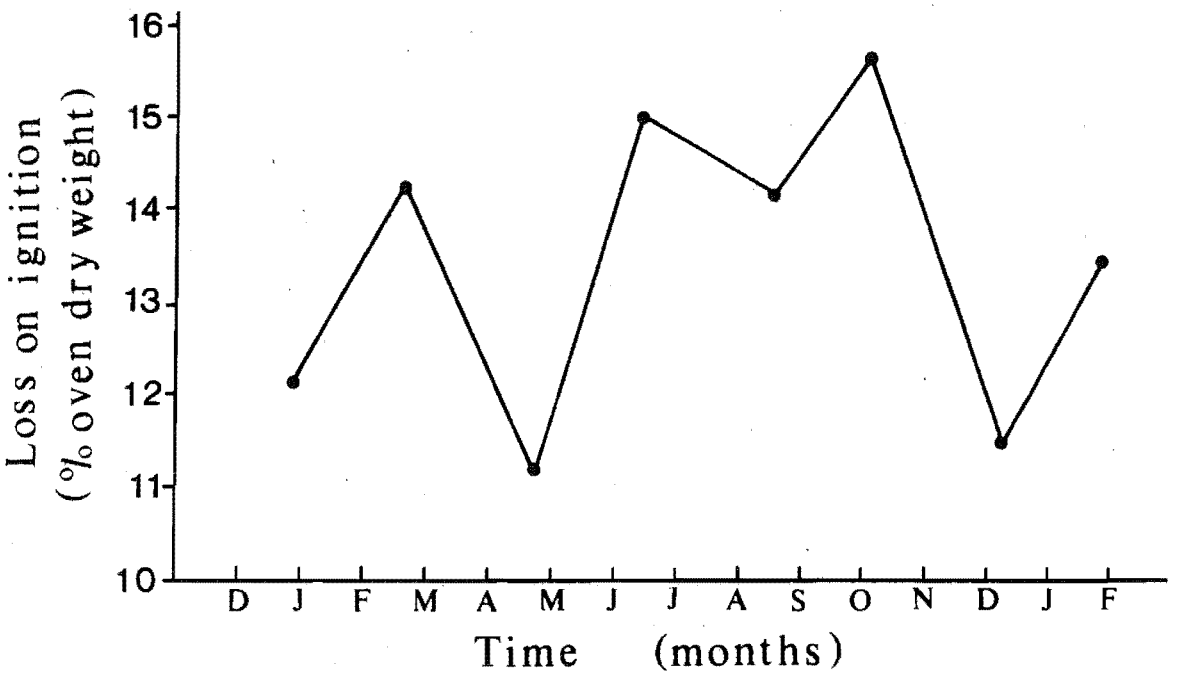

B
Suction tension (bars)
40
30
20
wc
10
fc
wc _ wilting coefficient
fc _ field capacity
5.4
4.2
3.0
Suction tension (pF)
10
15
20
25
30
Soil moisture
(% oven dry weight)

tolerated pressures above the permanent wilting point of plants (15 bars; pF 4.2). In general, as suction tensions approach the wilting point of plants, limiting effects on population growth can be expected. Thus for short periods during the summer at Broken River high soil moisture suctions probably contribute significantly to population regulation.

Organic matter: Seasonal trends of the organic matter composition of Broken River soil are illustrated in Figure 56. Microbial activity is the most important factor in the processes of humus formation, and the intensity of the activity is related to the hydrothermal factor (Kononova, 1966). Seasonal trends in moisture and temperature produce characteristic rythmical patterns of plant growth and activity of micro-organisms (Alexander, 1961). As with any biological phenomenon, the rate of decomposition of organic matter is governed by the factor which is most limiting (Kononova, 1966). Thus it is suggested that the winter peak shown in Figure 56 reflects a build-up of organic matter during a period of reduced microbial activity associated with low temperatures. As temperatures rise in spring the organic matter reserves are utilised in a bloom of micro-organism activity resulting in a downward trend in the graph. The increase of organic matter during summer may be interpreted as a reduction in microbial activity due to reduced soil moisture and/or an increase in plant material during the growing season, followed by a further reduction during autumn which probably coincides with a slowing down of plant growth and increased micro-organism activity.

Such a cycle is an over-simplication of the complicated interactions which may be involved, but it served to demonstrate that the effects of temperature and moisture on nematodes may be co-correlated with effects on food organisms. From the biology typical of these organisms it

FIGURE 56. Variation in loss on ignition.


Loss on ignition
(% oven dry weight)
16
15
14
13
12
11
10
D J F M A M J J A S O N D J F
Time (months)

seems reasonable to assume that the lag between cause and effect would be short. The consequent reduction of available food for nematodes dependent on bacteria, fungi, and algae could therefore contribute to the significance of the association of fluctuations of nematode numbers with temperature or moisture; the relative importance of each factor depending on season. This may be a contributing factor to the difference in the rate of response to environmental change apparent between ecological feeding groups (Tables 40, 42 and 43). For example, significant associations of numbers of bacterial feeders and filamentous feeders with temperature were recorded where no adjustment for a lag phase was made, and after adjustment for one sampling interval. The association of miscellaneous feeders and higher plant feeders with temperature was significant after adjustment for one, and two sampling intervals. Miscellaneous feeders are capable of surviving on a range of food types other than algae (see Chapter 5.2). The increased flexibility accompanying a wide diet would enable species in the group to tolerate environmental fluctuations within certain limits. Although plant growth is limited by fluctuations of the environment and physiological changes to the host may occur, some food would continue to be available to higher plant feeders. Under adverse conditions population growth may be limited and competition for feeding sites may intensify, but gross reductions of numbers would depend on the biology of individual species. That is, their generation time, the presence or absence of resistant stages in their life-history, their low lethal limits for survival and the specificity of their food requirements.

Because of the effects of aggregation, low frequency of occurrence, and sampling error, interpretation of fluctuations of specific populations is difficult. Considering

the three higher plant feeding species P. projectus, A. neozelandicus and T. maiakus which are among the most abundant species at Broken River, differential responses to environmental fluctuations can be seen (Tables 40, 42 and 43). The significant negative correlation of P. projectus with temperature and positive correlations with moisture and organic matter (Table 40) can be related to the presence of resistant fourth stage juveniles (see section 6.2.B.I). Numbers of P. projectus actually increased during the period of accumulating organic matter and increasing moisture coincident with a marked drop in temperature. Significant correlations of A. neozelandicus with environmental factors were recorded after adjustment for one and two sampling periods (Tables 42 and 43). T. maiakus on the other hand was positively correlated with temperature where no adjustment was made, and after adjustment for one sampling interval (Tables 40 and 42). It seems that T. maiakus is either shorter lived and/or less tolerant of extreme fluctuations of the environment than A. neozelandicus.

6.3.B.V Seasonal Effects

Seasonal effects of environmental factors on fluctuations of nematode numbers were implied in the previous section. In an attempt to identify such effects the data for ecological feeding groups was analysed after division into series based on season at two levels.

i) Samples from December 1968, February and December 1969, and January 1970 were combined as a summer series; April, June, August and October 1969 samples were included in a winter sample.

ii) Samples from December 1968, February, April and June 1969, were combined in a 'descending temperature' sequence; August, October, and December

1969 samples, and the January 1970 sample were included in an 'ascending temperature' series.

For each analysis data was tested as sampled, with an adjustment for one and then two sampling intervals. Transformation was carried out using Taylor's exact method according to the significance of the variance on the mean (Appendices V & VI).

Summer/winter series: From the results presented in Table 44 it is apparent that a significant positive association of total nematodes, and bacterial feeders occurs one sampling period earlier in summer than in winter. A similar trend is evident for higher plant feeders. This trend suggests that the lag phase may be longer in winter than in summer, which follows from previous discussion with regard to temperature effects on longevity of nematodes. The significant association of soil moisture and organic matter with total nematodes and higher plant feeders for summer samples where no adjustment for time was made, implicates these factors in effects on population growth during this period. Considering the correlation matrix for environmental factors (Table 45), it can be seen that soil moisture and organic matter are significantly correlated during summer, but during winter, when low temperatures were recorded, the order of the correlation is reduced. Hence it is suggested that while temperatures are consistently above the limits likely to become inhibitory to nematodes, soil moisture, in combination with effects on organic matter and related food influences, may limit growth of some nematode populations.

Interpretation of the associations of miscellaneous feeders and filamentous feeders is less clear. Short term exposure of miscellaneous feeders to unfavourable moisture and reduced food may be less critical because of their wide host range and long generation time. Further, it was

TABLE 44. Significance of the association between fluctuations in nematode numbers and fluctuations of soil moisture (M), soil organic matter (O), and soil temperature (T), for summer (S) and winter(W) samples, adjusted for 0, 1, and 2 sampling intervals.

Category	Season		Product-moment correlation coefficients			Multiple correlation coefficients			Order of entry
			M	O	T	1	2	3	
Total nematodes	S	0	0.3520*	0.4492**	-0.3335*	0.449*	0.506	0.514	O T M
		1	-0.1788	0.0545	0.3784*	0.378	0.407	0.423	T O M
		2	0.2766	0.1872	0.2044	0.277	0.432	0.441	M T O
	W	0	0.2644	-0.3125	-0.2432	0.313	0.467	0.499	O T M
		1	0.09241	-0.3313*	0.2572	0.331	0.372	0.485	O T M
		2	-0.3071	-0.0102	0.4392**	0.439	0.455*	0.461	T O M
Higher plant feeders	S	0	0.3553*	0.3467*	0.3467*	0.382	0.463*	0.481	T O M
		1	-0.1788	0.0892	0.0892	0.218	0.262	0.320	T O M
		2	0.3579*	0.2098	0.2098	0.358	0.423	0.428	M T O
	W	0	0.3049	-0.1573	-0.1573	0.306	0.399	0.434	T O M
		1	0.0778	-0.2967	-0.2967	0.297	0.330	0.404	O M T
		2	-0.1733	-0.1050	0.2644	0.264	0.266	0.267	T O M
Filamentous feeders	S	0	0.2185	0.2296	-0.0864	0.230	0.263	0.263	O M T
		1	0.0946	-0.0889	0.1631	0.163	0.233	0.269	T M O
		2	-0.3190	-0.1883	0.4259**	0.426	0.461*	0.461	T M O
	W	0	-0.1416	-0.2687	0.3410*	0.341	0.393	0.426	T O M
		1	0.0142	-0.0443	0.0034	0.044	0.049	0.227	O M T
		2	0.1969	0.2820	0.1924	0.282	0.315	0.346	O M T
Bacterial feeders	S	0	-0.1465	0.0885	0.3539*	0.354	0.397	0.409	T O M
		1	0.0801	0.0292	0.0515	0.080	0.119	0.119	M T O
		2	-0.1429	-0.0376	-0.2595	0.260	0.366	0.366	T M O
	W	0	0.4090*	-0.1433	-0.2889	0.409	0.458*	0.507	M O T
		1	0.0009	-0.2479	0.4926**	0.493*	0.525**	0.539	T M O
		2	-0.2108	0.3949*	0.1794	0.395	0.499*	0.532	O T M
Miscellaneous feeders	S	0	-0.1053	0.0208	0.1314	0.131	0.145	0.169	T M O
		1	0.1536	0.0782	0.0819	0.154	0.215	0.216	M T O
		2	-0.1475	-0.2102	0.0899	0.210	0.217	0.219	O M T
	W	0	0.0286	-0.0996	-0.1314	0.118	0.182	0.186	T O M
		1	0.2855	-0.3095	-0.1104	0.310	0.474*	0.474	O M T
		2	-0.1360	-0.0649	0.4717**	0.472*	0.500	0.504	T M O
Predators	S	0	-0.1217	0.1323	0.0500	0.132	0.247	0.248	O M T
		1	0.3583*	0.0688	-0.0926	0.358	0.376	0.377	M O T
		2	-0.0940	-0.01477	0.2303	0.230	0.249	0.252	T O M
	W	0	0.0392	-0.1558	0.1188	0.156	0.174	0.223	O T M
		1	-0.0325	0.1168	0.1006	0.117	0.182	0.183	O T M
		2	-0.1059	-0.0396	0.0697	0.106	0.107	0.108	M O T

r (35df)
0.05 = 0.325 *
0.01 = 0.418 **

R (35df)
0.05 = 0.445*
0.01 = 0.523**

TABLE 45: Significance of the association between fluctuations of soil moisture (M) organic matter (O) and soil temperature (T) during summer and winter.

		Product-moment correlation coefficients		
		M	O	T
M	Summer	1.0000	0.4719**	-0.3730*
	Winter	1.0000	0.2099	-0.5766**
O	Summer	0.4719**	1.0000	-0.2410
	Winter	0.2099	1.0000	-0.2849
T	Summer	-0.3730*	-0.2410	1.0000
	Winter	-0.5766**	-0.2849	1.0000

r(35df)

0.05 = 0.325*

0.01 = 0.418**

noted in Chapter 4.9, that some species tolerate partial desiccation in an inactive state.

Descending/ascending temperature series: In both series, significant positive correlations between total nematodes and temperature were recorded after adjustment for one and two sampling periods (Table 46). High negative correlations with soil moisture are evident in the descending series and are reflected in an increase in the order of the multiple correlation coefficients where the additive effects of temperature and soil moisture are involved. Higher plant feeders are correlated with temperature one sampling period earlier in the descending series. The influence of plant growth patterns may be involved in the increased lag during the spring/summer period (see section 6.4.B.IV.; comparison of tussock and inter-tussock faunas). The response of filamentous feeders to temperature changes is of the same order for both seasons. The relationships between temperature and bacterial feeders appears to be more critical during the period of rising temperatures. Stout (1960a) found that pseudomonads constituted about 50% of the strains isolated from the tussock rhizosphere. Psuedomonads grow well throughout the average range of monthly temperatures (Stout, 1965), which may delay the autumnal effect of temperature on the bacterial feeding fauna.

6.4. VARIATION IN THE VERTICAL PLANE

6.4.A Method

6.4.A.I Sampling

Four pits approximately one metre square were dug to a depth of about 50cm. Each pit was located so that one face was about 20cm from the base of a tussock. On seven occasions between February 1969 and January 1970 soil cores were drawn at depths of 0-2.5cm, 4.0-6.5cm, 7.5-10.0cm, and

TABLE 46. Significance of the association between fluctuations in nematode numbers and fluctuations of soil moisture (M), soil organic matter (O), and soil temperature (T) for samples grouped in descending (D) and ascending (A) temperature series, adjusted for 0, 1, and 2 sampling periods.

Category	Series		Product-moment correlation coefficients			Multiple correlation coefficients			Order of entry
			M	O	T	1	2	3	
Total nematodes	D	0	0.2045	0.0885	0.1066	0.205	0.427	0.463*	M T O
		1	-0.5971**	-0.2376	0.5970**	0.597**	0.642	0.645	M T O
		2	-0.6322**	-0.0918	0.6418**	0.649**	0.689	0.698	T M O
	A	0	-0.2717	-0.1268	0.1913	0.272	0.279	0.287	M O T
		1	-0.2718	-0.1941	0.6200**	0.620**	0.623	0.623	T O M
		2	0.0003	-0.0640	0.5364**	0.536**	0.625	0.625	T M O
Higher plant feeders	D	0	0.4291**	0.1751	-0.2102	0.429	0.588**	0.597	M T O
		1	-0.3737*	-0.1722	0.3662*	0.374	0.381	0.385	M O T
		2	-0.4593**	-0.1320	0.5324**	0.532**	0.534	0.535	T M O
	A	0	-0.1737	0.0631	0.0021	0.174	0.289	0.302	T M O
		1	-0.1234	-0.0119	0.2080	0.208	0.227	0.236	T O M
		2	0.0806	0.0343	0.3323*	0.332	0.465*	0.466	T M O
Filamentous feeders	D	0	-0.2437	-0.1389	0.3929*	0.393	0.466*	0.467	T M O
		1	-0.4301**	-0.1126	0.4529**	0.453*	0.456	0.456	T M O
		2	-0.0060	0.0170	0.3867*	0.437	0.523**	0.530	T M O
	A	0	-0.1120	0.0320	0.3133	0.313	0.367	0.368	T O M
		1	-0.2554	-0.1603	0.4586**	0.459*	0.461	0.462	T O M
		2	-0.0060	0.0170	0.3867*	0.387	0.464*	0.472	T M O
Bacterial feeders	D	0	-0.2022	-0.0620	0.1076	0.202	0.264	0.265	M T O
		1	-0.1614	-0.1851	0.2324	0.232	0.264	0.285	T O M
		2	-0.3247	0.2784	0.2964	0.325	0.502*	0.505	M O T
	A	0	-0.1334	-0.1123	0.5604**	0.550**	0.581	0.581	T O M
		1	-0.0683	-0.1966	0.6075**	0.607**	0.627	0.627	T M O
		2	-0.1613	-0.1368	0.1301	0.161	0.179	0.183	M T O
Miscellaneous feeders	D	0	0.2394	0.0396	0.1613	0.239	0.269	0.286	M T O
		1	-0.1900	0.0483	0.1756	0.190	0.190	0.190	M T O
		2	-0.5213**	-0.1488	0.5419**	0.542**	0.547	0.548	T M O
	A	0	0.0270	-0.0809	-0.1332	0.133	0.205	0.208	T O M
		1	-0.1518	-0.2670	0.5162**	0.516*	0.543	0.566	T M O
		2	-0.2751	-0.3087	0.4303**	0.430*	0.450	0.451	T O M
Predators	D	0	-0.0379	0.0309	0.0626	0.063	0.081	0.091	T O M
		1	-0.0273	0.1662	0.1966	0.197	0.392	0.453*	T M O
		2	-0.3121	-0.1264	0.2963	0.312	0.315	0.317	M O T
	A	0	-0.1441	-0.1134	0.0956	0.144	0.146	0.147	M O T
		1	0.0483	-0.1297	0.1528	0.153	0.223	0.229	T M O
		2	-0.0841	-0.1555	0.0947	0.156	0.158	0.162	O T M

r (35df)
0.05 = 0.325*
0.01 = 0.418**

R(35df)
0.05 = 0.445*
0.01 = 0.523**

30-32.5cm, from the rhizosphere of a fescue tussock, and from a position in the inter-tussock zone approximately 25cm away. Forty-six to 60 days elapsed between samples. Following each sampling a slab of expanded polystyrene about 250 x 40 x 2.5cm was placed against the sampled face and the pits were refilled.

By August, four series of samples had been taken from each station. Although tussock crowns are large, continued damage by coring through the base of the plants was considered to be inadviseable. Subsequent samples were therefore taken from four new pits. To maintain continuity between the two series, cores to a depth of 10cm were drawn from the tussock and inter-tussock zones of both at the sampling prior to the change-over.

6.4.A.II Extraction and measurement of environmental parameters

From each core 10cm in length, 2.5cm or the equivalent volume was retained for measurement of soil moisture and organic matter by the methods described in section 6.2.A.III. The remaining four 7.5cm samples from each depth were bulked and processed following the method described in section 6.2.A.II, and Chapter 2. Soil temperatures at the various depths were recorded at each sampling using a mercury-in-glass thermometer.

6.4.A.III Assessment of the soil micro-flora

For February, April, June and November samples, soil suspensions were prepared from 10g of soil for each depth and numbers of bacteria and fungi present estimated using the conventional dilution plate method. Two plates for dilutions of 1/1,000, 1/10,000 and 1/100,000 were prepared for bacteria counts and a further two at dilutions of 1/100, 1/1,000, and 1/10,000 for counts of fungi.

Bacteria counts were made on the basis of colony growth on Difco nutrient agar after incubation of the cultures for 48 hours and 96 hours at 24°C. Fungi were grown on Martin's medium (Martin, 1950) incubated at 24°C and colony counts recorded after 48 hours and five days.

6.4.B Results and discussion

6.4.B.I Variation with time and depth

From the results illustrated in Figure 57 it can be seen that the greatest concentration of nematodes in the tussock and inter-tussock rhizospheres occurs in the top 10cm of soil. Seasonal fluctuations are most evident in samples from this zone: a reduction of numbers during the autumn-winter period (April and June samples) and a recovery during spring-summer is apparent. The autumn decline and the spring increase in numbers both occurred earlier in the inter-tussock zone than under the tussocks. At the August sampling, prior to a change of site in September, numbers of nematodes to a depth of 10cm were recorded for both locations (Table 47). The increased numbers of nematodes at 0-2.5cm and 4.0-6.5cm in the inter-tussock zone during September can be ascribed in part to the effects of aggregation incurred in the site change, but additional factors which may be implicated are discussed in the sections below.

The histograms show that faunal numbers exhibit a seasonal pattern with depth. An increase in total numbers with depth to 10cms is evident in both zones in the February sample. The pattern is less regular throughout the winter period, but during spring, numbers in the top 10cms of the inter-tussock zone are inversely related to depth. At 15.0-17.5cm, fluctuations are slight and appear to lag behind those of nematodes in the upper strata.

FIGURE 57. Variation of the nematode fauna in the tussock and inter-tussock rhizospheres with depth and time.

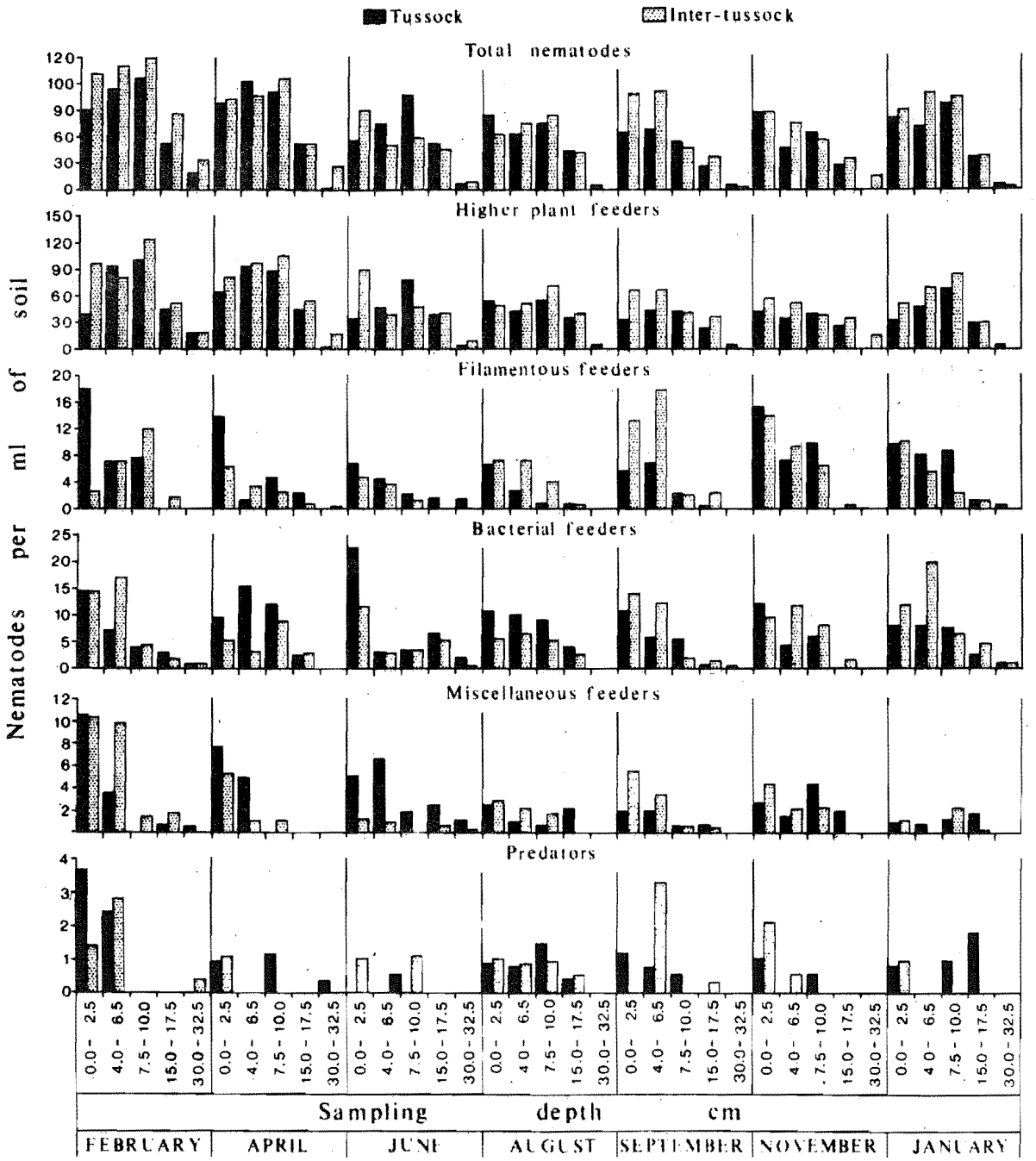

Tussock
Inter-tussock
Total nematodes
Higher plant feeders
Filamentous feeders
Bacterial feeders
Miscellaneous feeders
Predators
Nematodes per ml of soil
0.0 - 2.5
4.0 - 6.5
7.5 - 10.0
15.0 - 17.5
30.0 - 32.5
Sampling depth cm
FEBRUARY
APRIL
JUNE
AUGUST
SEPTEMBER
NOVEMBER
JANUARY

TABLE 47: Numbers of nematodes from the top 10cm of soil from two pit locations.

Site	Category	Nematodes per ml of soil	
		Tussock	Inter-tussock
Old	Total nematodes	72.6	62.1
	Higher plant feeders	54.2	45.1
	Filamentous feeders	4.8	7.1
	Bacterial feeders	7.5	4.8
	Miscellaneous feeders	0.6	1.8
	Predators	2.8	1.5
New	Total nematodes	66.7	81.9
	Higher plant feeders	48.7	54.3
	Filamentous feeders	5.1	7.9
	Bacterial feeders	6.6	7.4
	Miscellaneous feeders	2.3	3.1
	Predators	0.0	3.4

Previous authors have observed similar seasonal distribution patterns in the vertical plane (e.g. Lellakova-Duskova, 1964; Yeates, 1968a). Wallace and Greet (1964) suggested that such vertical distribution patterns may result from nematode migration to particular zones of the soil under the influence of environmental stimuli, and from differential rates of reproduction at more favourable depths. Both factors are probably operative in the above cycle, the relative importance of each depending on depth and season.

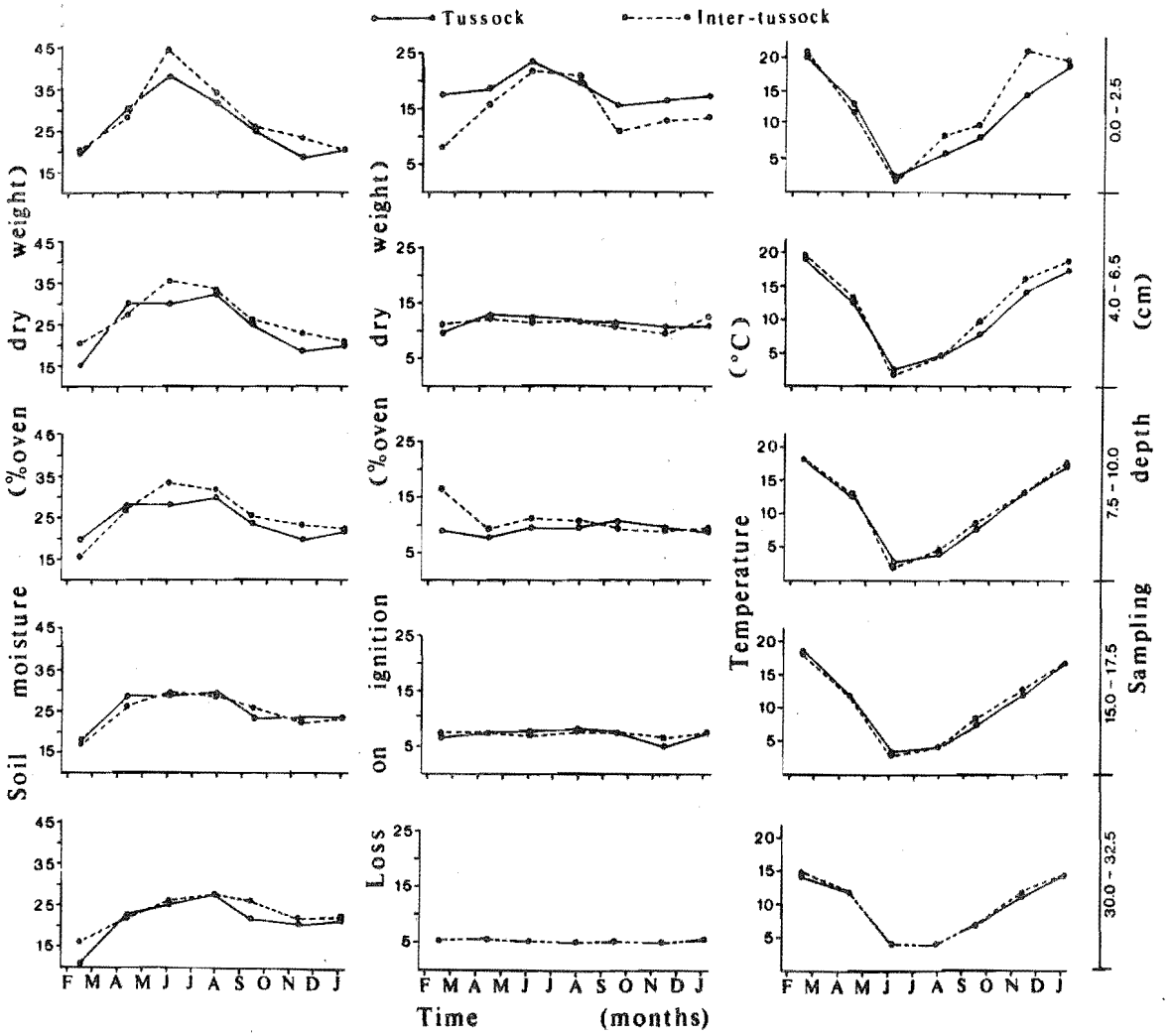
Seasonal trends of higher plant feeders in the inter-tussock zone follow the general trend of total nematodes.

The contribution of filamentous feeders and bacterial feeders to the increase in total numbers at 0-2.5cm and 4.0-6.5cm in the September sample is proportionately greater. Similarly, filamentous feeders contribute largely to the increase of total numbers recorded in the top 10cm of the tussock rhizosphere in November. Development of higher plant feeders was not marked until January, at which time numbers increased with depth to 10cm. Irregularities evident in the histograms indicate that the distribution of nematodes is aggregated in the vertical plane, particularly where numbers are low (e.g. miscellaneous feeders and predators). Seasonal trends in these groups cannot be ascertained from the present data.

6.4.B.II Variation of environmental factors

Variation of environmental factors with season is summarised in Figure 58. Maximum variation in both tussock and inter-tussock rhizospheres occurs in the top 10cm of soil which coincides with the zone of maximum fluctuation of nematode numbers. Fluctuations at stations above 10cm are most severe in the inter-tussock zone. Previous workers have recorded localised climatic modifications associated with tussock plants (e.g. Gradwell, 1954, 1955; Scott, 1962). Gradwell (1954) reported that July temperatures were higher under tussocks at depts of 2.5cm and 7.5cm than in the inter-tussock soil. Later, Gradwell (1955) showed that the diurnal range of soil temperatures during October was considerably reduced under the tussock crown. In the present study, minimum temperatures were lower in the inter-tussock zone, indicating a buffering action of the tussock (Figure 58). Temperatures during autumn follow a similar trend in both zones, but the diurnal variation in the inter-tussock rhizosphere may be more severe (see Gradwell, 1954, 1955). During spring the inter-tussock soil temperatures for

FIGURE 58. Variation of environmental factors in the tussock and inter-tussock rhizosphere with depth and time.


Tussock
Inter-tussock
Soil moisture (% oven dry weight)
(% oven dry weight) loss on ignition
Temperature (°C)
Sampling depth (cm)
0.0 - 2.5
4.0 - 6.5
7.5 - 10.0
15.0 - 17.5
30.0 - 32.5
F M A M J J A S O N D J
Time (months)

samples above 6.5cm are higher than those for corresponding depths in the tussock rhizosphere. Although temperatures were recorded between 1pm and 3pm, and a bias in favour of high inter-tussock temperatures could be expected; considerable diurnal variation is indicated.

6.4.B.III Variation of the micro-flora

Maximum numbers of bacteria were recorded in November and minima occurred in June (Table 48). Isolates of fungi were lowest in June, but maxima in the tussock rhizosphere were recorded in February; maximum numbers were recorded in April for the inter-tussock samples. Populations of both fungi and bacteria were consistently greater in the top 2.5cm of soil, which coincides with the zone of maximum organic matter concentration (Figure 58), decreasing with depth.

In discussing the seasonal effects of environmental factors on fluctuations of nematode numbers it was suggested that soil moisture, in combination with effects on organic matter and related food influences, may limit nematode population growth during summer (section 6.2.B.V). The optimum soil moisture levels for activities of aerobic bacteria is often at 50-75% of the soil moisture holding capacity (Alexander, 1961), which approximates the range of 40-60% which has been suggested as favourable for the activity of nematodes (Stolzy and Van Gundy, 1968). Clark, F.E. (1967) notes that at moisture tensions of 3 bars of higher, bacterial activity in soil becomes reduced, and markedly so at the permanent wilting point of plants (15 bars). Field capacity of 'average' top soil from Broken River occurred at about 30% moisture content on an oven dry weight basis (Figure 55). Soil moisture levels rarely exceeded the lower favourable limits for growth of bacteria but at moisture contents of less than 20% some reduction of

TABLE 48: Plate counts of bacteria from soil samples in the vertical plane of tussock (T) and inter-tussock (I) rhizospheres.
($\times 10^5$/g oven dry soil)

Sample depth cms	Time of sample							
	February		April		June		November	
	T	I	T	I	T	I	T	I
0.0-2.5	280	206	205	155	108	188	386	326
4.0-6.5	42	82	58	91	30	56	124	208
7.5-10.0	92	122	51	32	32	66	116	126
15.0-17.5	30	46	48	40	24	34	45	72
30.0-32.5	10	16	8	1	5	8	2	4

TABLE 49: Plate counts of fungi from soil samples in the vertical plane of tussock (T) and inter-tussock (I) rhizospheres.
($\times 10^4$/g oven dry soil)

Sample depth cms	Time of sample							
	February		April		June		November	
	T	I	T	I	T	I	T	I
0.0-2.5	59	33	51	39	41	17	56	31
4.0-6.5	22	19	38	31	11	6	24	23
7.5-10.0	14	10	15	10	5	2	11	5
15.0-17.5	11	9	6	8	3	1	4	5
30.0-32.5	1	1	1	3	2	3	4	2

development could be expected. Robinson and MacDonald (1964) recorded a reduction of numbers of bacteria from November to December in tussock soils about four kilometres away from the Broken River site. Similarly, lower numbers were recorded in February compared with November in the present isolations. Griffin (1963) noted that nearly all fungi would be able to grow unimpeded by reduced hydraulic conductivity of soil throughout the suction range of pF 0-4.2, although the tolerance of different species may vary. A reduction of fungi isolations was not evident during summer in the present series (Table 49). If the reduction of numbers of bacteria is also indicative of a reduction of availability, then it is probable that food may have a more significant limiting effect on populations of bacterial feeding nematodes than filamentous feeding forms. But if food was the only limiting factor to bacterial and filamentous feeding nematodes it is reasonable to expect the distribution of nematodes to follow a similar pattern to the distribution of these organisms. Figure 57 shows that distributions of filamentous feeders and bacterial feeders do not exhibit a 'proportionate' relationship to the numbers of micro-organisms at various depths (Tables 48 and 49). Populations are largest in samples from the top 10cm of soil, but numbers in the 0-2.5cm zone do not reflect the abundance of micro-organisms at this depth. The extreme short term fluctuations of physical factors which occur in the surface layers of the soil may therefore be the major regulating factor.

6.4.B.IV Comparison of seasonal trends of the tussock and inter-tussock nematode faunas.

To minimise the effect of aggregation and differential fluctuations of the fauna with depth, samples from the

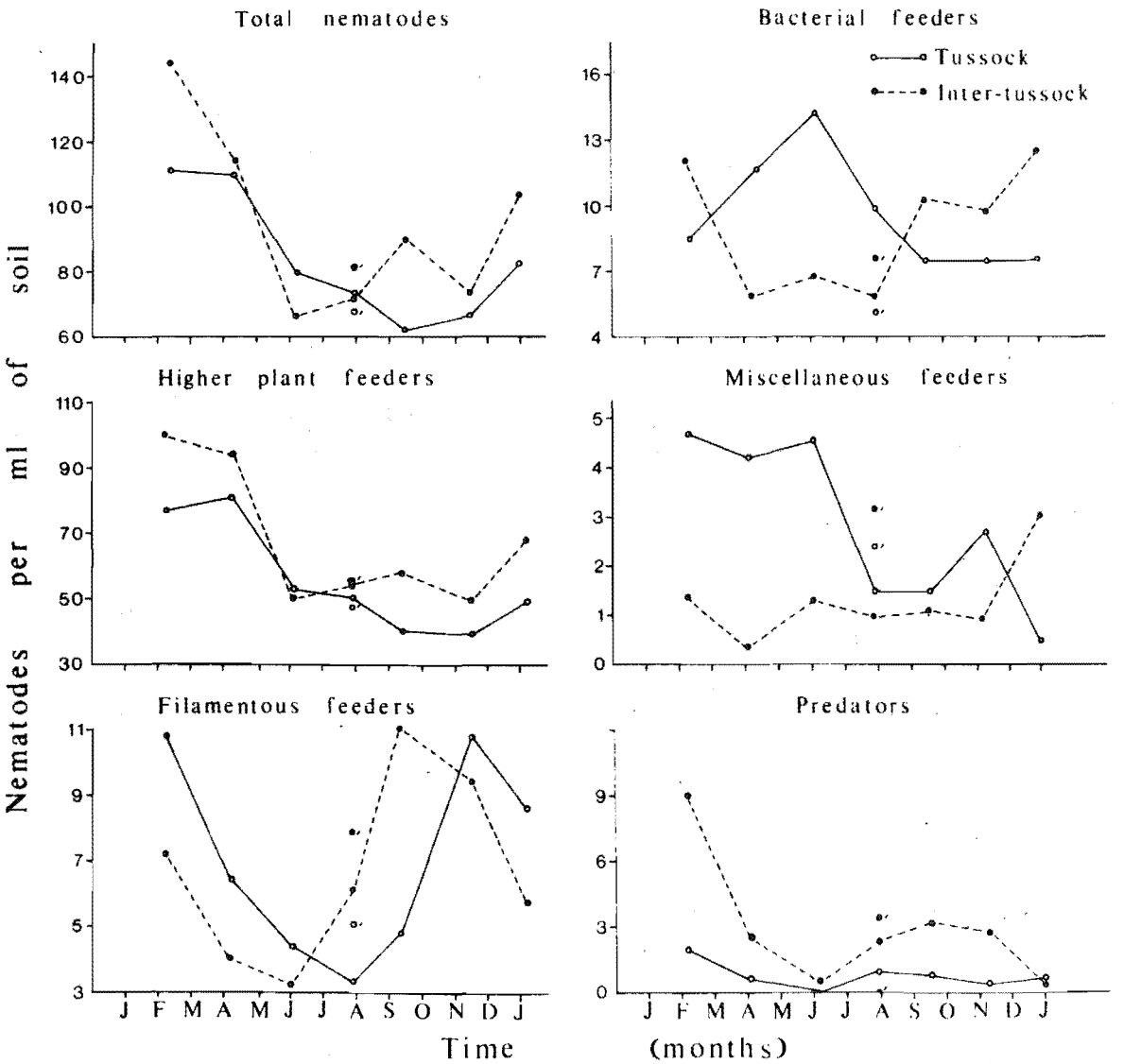
0-10cm zone were considered as replicates and the mean numbers for each zone were calculated. Samples from the horizontal plane (section 6.3) were taken to a depth of 10cm and an average 7.5cm sample processed. Hence the mean of the top three samples from the vertical series approximates a horizontal sample and enables the comparison of both series. Fluctuations of total nematodes in the two tussock series show similar trends with a single peak occurring in summer to autumn (compare Figures 59 and 47). An earlier reduction of the inter-tussock fauna is evident during autumn and winter and a minor peak is shown during September (Figure 59).

Decreasing temperature was shown to be correlated with declining nematode numbers in section 6.3.B.IV. Further, the diurnal range of soil temperature is less under tussocks (section 6.3.B.II). The modification of soil temperature profiles by thermal insulators at the surface reduces the influence of solar radiation (Raney, 1965). Tussock plants possess a dense crown of tillers at the base which may buffer temperature extremes during autumn-winter, and delay the warming of soils in the spring-summer period. Hence it is suggested that the exposure of nematodes to lower daily minimum temperatures earlier in autumn and to higher daily maxima in spring contributes to the reduction of the lag interval in the inter-tussock zone.

The apparent September increase in the inter-tussock rhizosphere may be interpreted in part from the effects of the change of site (Figure 59). However, the inter-tussock zone is dominated by the introduced grasses sweet vernal and browntop (Chapter 2). Lambrechtson (1968) emphasised that sweet vernal is characterised by a flush of root growth in early spring, before leaf growth is initiated. The importance of root exudates in stimulating microbial activity is well established (Rovira, 1965b). A reduction

FIGURE 59. Variation of mean numbers of nematodes in the 0-10cm zone of the tussock and inter-tussock rhizospheres.

●′ Indicate numbers recorded
o′ from the new site positions one sampling period prior to the changeover.


Total nematodes
Bacterial feeders
Tussock
Inter-tussock
Higher plant feeders
Miscellaneous feeders
Filamentous feeders
Predators
Nematodes per ml of soil
J F M A M J J A S O N D J
Time
(months)

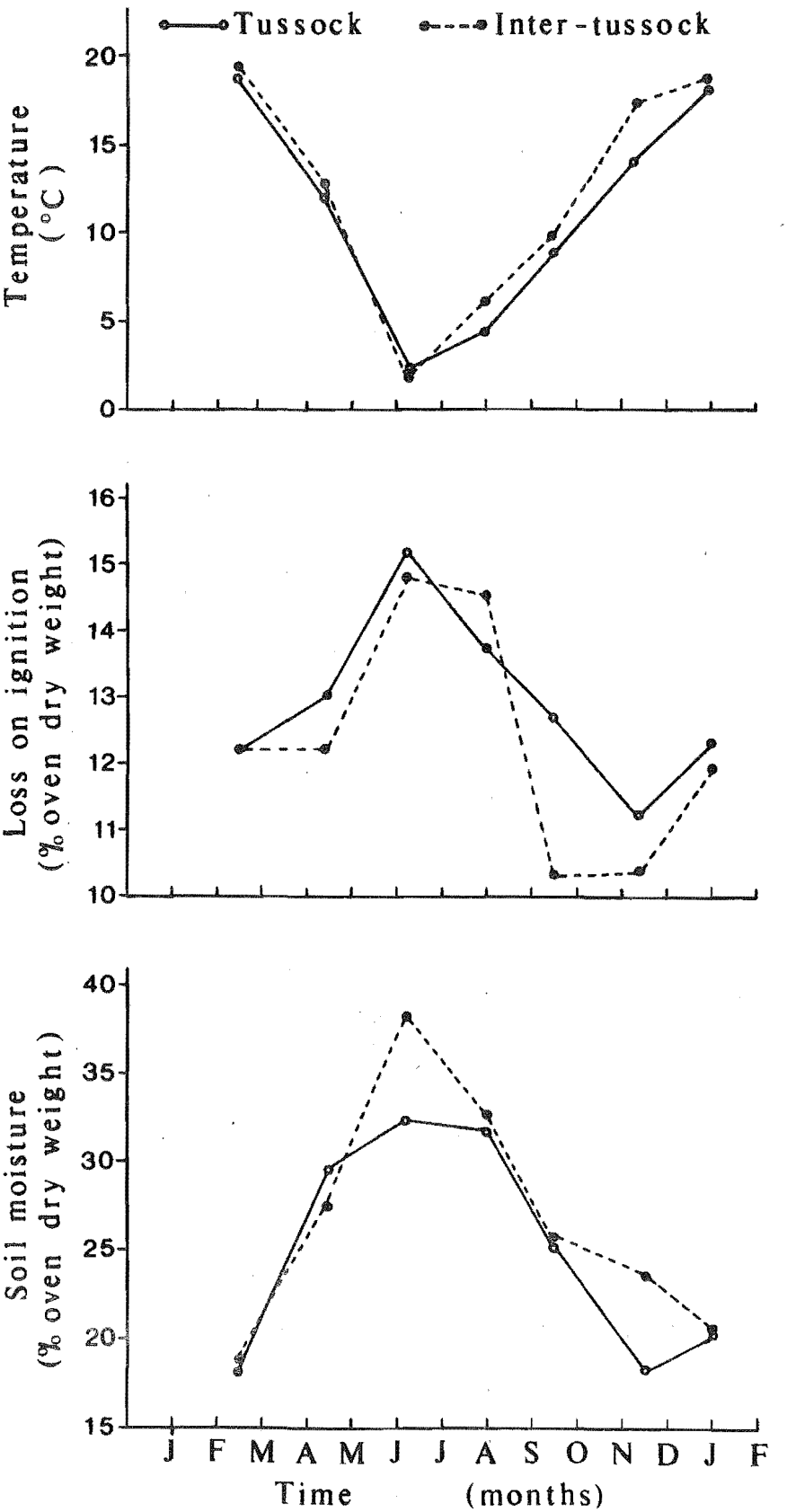
of soil organic matter in the inter-tussock zone for the September sample indicates that microbial activity has increased (Figure 60). Root exudates are also known to stimulate the activities of some nematodes (e.g. Seinhorst, 1961; Wallace, 1963). Further, it has been noted that some nematodes are capable of surviving adverse conditions in a quiescent or anabiotic state (section 6.2.B.IV). Dao (1970) reported that nematodes from temperate and mountain climates may survive spells of very low temperatures and resume activity at about 10°C. Thus it is suggested that the slight increase of nematode numbers in the inter-tussock rhizosphere relative to the tussock rhizosphere evident in September samples may be associated with a more rapid increase in temperature due to less efficient thermal insulation in the top few centimetres of soil in the inter-tussock zone, together with the stimulation of nematode activity by early root growth of inter-tussock plants. A 'resumption of activity' rather than an increase in numbers could explain the absence of a lag effect.

6.5 GENERAL DISCUSSION

Numbers of nematodes per square metre in the top 10cm of soil under fescue tussock plants fluctuated from 7.7 millions to 10.6 millions over a full years cycle. Minimum numbers occurred in winter, rising in late spring, with late summer maxima and a decline during autumn. A similar trend was shown in the inter-tussock zone but more severe fluctuations involving greater than two-fold increases in numbers were observed.

Comparison of population trends and nematode densities between workers is of doubtful value because of the variation between sites, and because of the variety of sampling and extraction methods used. However, it is interesting to note that seasonal variation of nematode densities at Broken River are less severe than those reported by many previous

FIGURE 60. Variation of environmental factors in the 0-10cm zone of the tussock and inter-tussock rhizospheres.


Tussock
Inter-tussock
Temperature (°C)
0
5
10
15
20
Loss on ignition (% oven dry weight)
10
11
12
13
14
15
16
Soil moisture (% oven dry weight)
15
20
25
30
35
40
J F M A M J J A S O N D J F
Time (months)

workers (e.g. Seidenschwarz, 1923; Burkhalter, 1928; Lellakova-Duskova, 1964; Winslow, 1964; Zuckerman, Khera and Pierce, 1964; Banage, 1966).

Interpretation of fluctuations of nematode numbers was complicated by the aggregated distributions of nematodes within the fauna and by the presence of a lag phase between environmental changes and nematode trends. By taking large numbers of samples and including specific populations in functionally related groups (ecological feeding groups) distribution problems were minimised. However, the proportion of unexplained variation in the regression analyses is very high. The coefficient of determination (the square of the correlation coefficient) is a measure of the variation in a dependent variable which is explained by the independent variable (Steel and Torrie, 1960). With reference to Tables 40, 42 and 43 it can be seen that the proportion of the variation explained by the independent variables does not exceed 50% in any of the analyses. Yeates (1968b)considered the population dynamics of sand-dune nematodes in detail. From his data it can be seen that even in a'relatively simple' habitat, the amount of variation explained (R^2) by measured environmental factors only exceeded 50% for two species.

The sources of variation are complex; many have been indicated in discussion throughout the chapter. For example, by limiting environmental observations to only three parameters, many interactions are not considered. Ecological feeding groups consist of several species, each of which may exhibit different responses to environmental fluctuations in particular circumstances. This is very apparent in considerations of lag effects. The duration of the lag phase varies not only between species, but between seasons for any one species. Hence considerations of nematode population dynamics would ideally involve considerations

of specific populations for which detailed biological information was available. However, at the species level, the sampling problems inherent in aggregated populations precluded such a approach. Further, biological studies on all of the species present would necessitate several years work. But, it has been clearly shown in the present study that logical discussion of trends of ecological feeding groups depends on some understanding of the biology of main species comprising these groups.

Temperature is considered to be the most limiting factor to the development of the nematode fauna at Broken River. Temperature is critical during autumn, winter and spring and its effects are manifest through a direct action on nematodes, through limiting population replacement, and through associated effects on the soil fauna and flora. Soil moisture is probably most limiting to nematodes and to the availability of food in the top soil during the summer months, but interpretation of food influences is complicated where 'abundance' rather than 'availability' is assessed (e.g. micro-organisms associated with feeding of filamentous feeders and bacterial feeders).

Vegetation was shown to be important in quantitative effects on seasonal trends of nematodes. The lag phase was reduced in samples from the inter-tussock zone and further, where a single late summer peak was shown for nematodes from tussock samples, a small second peak during spring occurred in the inter-tussock zone. A buffering action of tussocks on fluctuations of environmental factors and differential plant growth patterns is implicated.

It is suggested that some increase of nematode numbers after prolonged exposure to winter conditions may reflect an 'activation' of quiescent animals through temperature increase, plant root growth and stimulated microbial activity. If this is a real effect, it follows that minimum

numbers recorded during winter may be an under-estimate of the actual fauna. To clarify this suggestion, more detailed information on the tolerance of each species to winter conditions is required.

7. PLANT PATHOLOGY

7.1 INTRODUCTION

Plant pathology is concerned with the health and productivity of growing plants. Every plant has a genetically determined production potential which would be expressed under conditions optimal for its development. In nature such conditions rarely occur. Several factors may be operative in preventing the realization of optimum productivity, and plant disease is one of the most important of these.

Nematodes have long been recognised as incitants of plant disease: by their feeding processes they may induce plant damage, either directly,or in association with other soil micro-organisms. In the present study several of the nematode species isolated have been demonstrated as parasites of plant roots and root hairs (Chapters 3 and 4). These nematodes frequently constituted more than 50% of the processed samples. Most of the species involved were ecto-parasites, that is, they do not normally enter the root tissue. However, despite the occurrence of large numbers of phytophagous nematodes no obvious symptoms of ill-thrift, abnormal growth, or plant decline were observed in the field. But soil-borne pathogens are known to cause crop loss without the expression of dramatic symptoms above the ground and in many instances these pathogens may be of importance by the very nature of their widespread occurrence and continuous debilitating effect on plants. It has been suggested that even in mono-specific plant stands a loss of 15% frequently occurs before a disease condition may be recognised (Chester, 1951).

Having demonstrated the prevalence of ecto-parasitic nematodes in the soil at Broken River, and confirmed the wide host range of the main species involved, it seemed possible that a relatively uniform growth retardation could occur undetected. As it was not possible to consider all of the potentially pathogenic species involved, two of the most common species, P. projectus and A. neozelandicus were selected for experiments to investigate the effects of nematode parasitism on plant growth. Relevant literature pertaining to both species was reviewed in Chapter 3 in conjunction with considerations of their respective host ranges and biology. For reasons of simplicity, two ecologically important plant species of the Broken River flora, F. novae-zelandiae (fescue tussock) and A. odoratum (sweet vernal), were used as indicator species. The physiognomically dominant fescue tussocks are important in providing shelter for seedling growth; in buffering extreme environmental changes, and in producing a supply of humus which promotes moisture conservation and reseeding (Cockayne, 1910; Sewell, 1947). It has been suggested that continued use of the montane tussock grassland for pastoral purposes depends on the preservation of the tussocks (Zotov, 1938; Sewell, 1947) The exotic grass sweet vernal contributes largely to the sward between the tussocks. It is an intermediate plant with respect to leaf length, tillering frequency and habit, characterised by early spring growth and the ability to act as a colonizer (Lambrechtson, 1968). It is readily grazed by stock (Milton, 1953) particularly in spring (Cockayne, 1920), autumn and winter (Davies, 1925), but is neglected in summer (Cockayne, 1920; Davies, 1925).

7.2 GENERAL METHOD

Experiments were carried out using plants grown under green-house conditions and plants grown in temperature controlled rooms.

Potting medium: Top soil from the Broken River site was used as the potting medium in all of the trials. Sterilization was effected by moist heat at 212°F for 30 minutes (Baker, K. F, 1957).

Seed treatment: Seed was harvested from Broken River, and prior to sowing was surface sterilised by immersion in a 4:1 solution of 0.01% mercuric chloride and 95% ethyl alcohol for 10 minutes (Wood, 1966). Germination of F. novae-zelandiae seed was improved by a four week incubation period at 4°C, under moist conditions (Appendix VII).

Plant establishment: Seedlings were established in plastic bags supported by wax pots. Both the pots and bags were perforated from the bottom to facilitate drainage. When re-potting, the plastic bags and seedlings were lifted free, the bags removed with minimal root disturbance, and the plants and soil transferred to a larger container.

Watering: In green-house trials a system of bottom watering incorporating deep wet-sand trays was used for small pots, and an overhead sprinkling system for large pots. Plants were fed weekly with nutrient solution (N:P:K:, 32:12:12). Plants maintained under closely controlled conditions in growth rooms were overhead watered every second day, and unless otherwise stated were fed twice weekly with nutrient solution.

Standardisation of soil moisture: Soil moisture was determined as a percentage of the oven dry weight. Four pots for each treatment were taken at random and the amount of water required to bring them to a standard weight was measured. The mean volume estimated from the

records was applied to the rest of the pots in respective treatments. An estimate of variation was obtained by determining the weight loss on drying for sub-samples taken at random.

Estimation of water potential: Soil samples of known moisture content (percent of the oven-dry weight) were prepared. Total suction potential, that is including both matric and osmotic suction components (Williams, 1968), was measured using a thermocouple psychrometer (Galbreath, 1970), and the moisture content re-estimated by drying (Figure 61).

Growth room environment: Ambient temperature and humidity were recorded continuously on hydro-thermographs. Soil temperatures were measured twice-weekly with a mercury thermometer inserted to a depth of approximately 3.0cm. The growth rooms were illuminated by a bank of 50, 80 watt internal reflecting fluorescent tubes, supplemented by 52, 15 watt tungsten bulbs. Light intensity was 1200 ft. candles at approximately one half the foliage height from the soil surface for large tussocks and 950ft candles at the level of sweet vernal seedlings. A day length of 16 hours was maintained throughout the experiments.

Inoculation: Plants were inoculated by pipetting nematode suspensions into a number of channels in the soil close to the crown. Nematodes were surface sterilised by immersion in a 0.1% W/V solution of methoxy ethyl mercury chloride (6% mercury) for 15 minutes, and rinsed in sterile distilled water.

Population levels: Soil samples for the assessment of nematode populations in inoculated pots were taken through the centre of plants using a corer of 2.5cm diameter. Estimates were made following extraction by the methods described in Chapter 2.

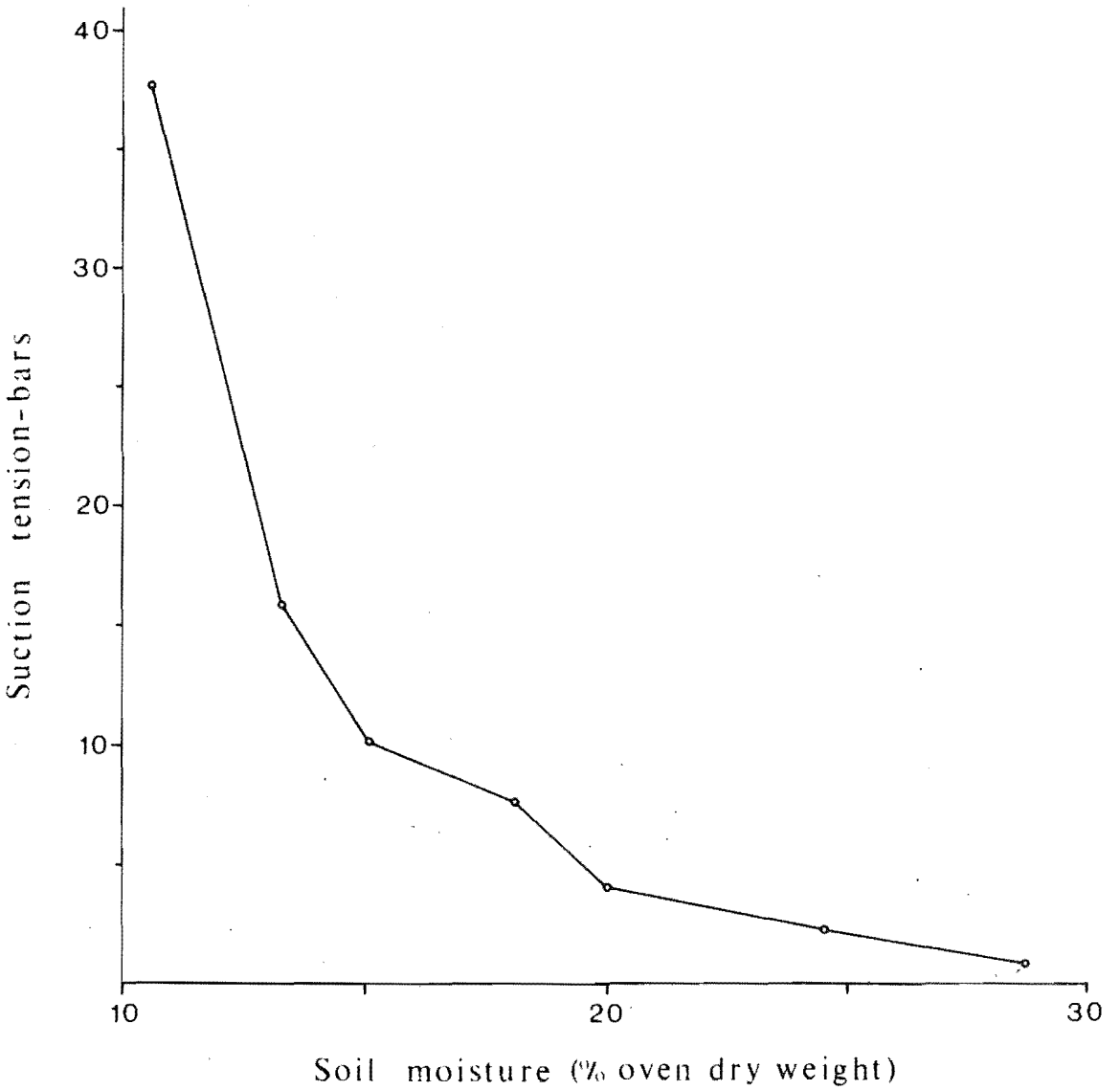


Figure 61. Relationship between soil moisture (% oven dry weight) and suction tension.

Dry matter production of foliage and roots: On completion of an experiment, plant foliage was removed by cutting at the level of the soil surface. The pots were then immersed in water for 24 hours before the soil was gently manipulated under water and the roots separated by hosing over sieves. Dry weights were recorded after drying for 72 hours at 65°C.

Analysis of data: The significance of differences between treatments was tested using Wilcoxon's rank-sum test for identical populations, sensitive to unequal locations (Bradley, 1968).

7.3 EFFECT OF ECTO-PARASITIC NEMATODES ON PLANT GROWTH

7.3.A Inoculated F. novae-zelandiae

METHOD

Approximately 12 fescue tussock seeds were sown in each of 24 pots. When the average height of the seedlings was about 5.0cm they were selectively thinned to three per pot on the basis of the eveness of shoot production. Seventeen weeks after sowing each group of three plants was replanted in about 4.0Kg of soil in a 17.5 x 20.0cm container. Three weeks later eight containers were inoculated with 100 surface sterilised P. projectus, eight with 100 surface sterilised A. neozelandicus and eight with sterile water. Placement of the plants in the green-house was determined using a table of random numbers and positions were changed at weekly intervals. Nematode population levels and dry matter production of roots and shoots were determined 28 weeks after inoculation (48 weeks after sowing).

RESULTS AND DISCUSSION

Nematode populations determined from an aliquot from four samples for each species were 85 P. projectus per ml of soil, and nine A. neozelandicus per ml.

Analysis of the results presented in Table 50 showed that inoculation of F. novae-zelandiae with ecto-parasitic nematodes did not produce significant effects on dry matter production of roots and shoots. However, the fact that seedlings were nematode-free during establishment together with the lag phase involved in the increase of nematode populations, meant that unaffected growth occurred for a considerable part of the trial. As fescue tussocks are relatively slow growing plants, small differences due to subsequent nematode parasitism may have been masked by between-plant variability.

7.3.B Effects on plants grown in infested soil

In the field, young plants are exposed to nematode parasitism at, or soon after germination. During the early stages of plant growth the root system develops rapidly, the rate increasing to a maximum a few weeks after germination, at which stage it begins to decrease (Troughton, 1957). If seedling growth is to proceed unchecked, a continuous supply of water and nutrients is required and this depends on root development (Asher and Ozanne, 1966). Detrimental effects of nematode parasitism on plant growth could be expected to be most severe during the early growth phase.

METHOD

A. odoratum: Surface sterilised seed was sown in 7.0 x 9.5cm pots containing non-infested soil and soils infested with populations of P. projectus (127 per ml),

TABLE 50. Dry matter production of green-house grown F. novae-zelandiae plants inoculated with P. projectus, A. neozelandicus and sterile water.

Treatment		Dry matter production grams oven dry weight			Ratio of root:shoot
		Shoot	Root	Total	
Plants inoculated		73.83	26.07	99.90	0.353
with P. projectus		22.62	15.43	38.05	0.682
		62.16	30.17	92.33	0.485
		33.14	14.61	47.75	0.441
		44.16	30.31	74.47	0.686
		48.53	24.18	72.71	0.498
		51.94	29.32	81.26	0.564
		31.62	17.14	48.76	0.542
	Mean	46.00	23.40	67.40	0.531
Plants inoculated		51.90	16.55	68.45	0.319
with		53.85	25.58	79.43	0.475
A. neozelandicus		43.50	15.90	59.40	0.365
		27.42	14.13	41.55	0.515
		31.18	25.21	56.39	0.808
		55.13	22.61	77.74	0.410
		56.01	24.12	80.13	0.431
		28.19	14.18	42.37	0.503
	Mean	43.40	18.53	63.17	0.478
Plants inoculated		26.00	14.07	40.07	0.541
with		23.22	19.85	43.07	0.855
sterile water		60.12	35.85	96.57	0.596
		38.90	23.32	62.22	0.599
		48.45	20.91	69.36	0.432
		25.36	17.14	42.50	0.676
		27.81	15.01	42.82	0.540
		59.15	31.15	90.30	0.527
	Mean	38.61	22.16	60.85	0.596

and A. neozelandicus (11.5 per ml). Non-infested soil was obtained from pots which had previously supported fescue tussocks sown in sterilised soil. Three weeks after sowing seedlings were thinned to three per pot on the basis of shoot growth. Ten containers were for each treatment maintained in a growth room at 16°C for seven weeks before plant dry matter production was determined.

F. novae-zelandiae: Because of the slow establishment of F. novae-zelandiae from seed, ten week-old seedlings of the same size were transplanted into 9.5 x 10.0cm pots containing the infested and non-infested soils. Ten replicates were prepared for each of the three treatments. Plants were harvested after 12 weeks at 16°C.

RESULTS AND DISCUSSION

Environmental variation: Air temperature, 11.5-19.5°C; soil temperature, 14.5-18.5°C (mean, 17°C); soil moisture, 27-40% (mean, 31%); relative humidity, 80-100%

Total plant dry weights and dry weights of roots and shoots for A. odoratum and F. novae-zelandiae are presented in Tables 51 and 52. Analysis revealed no significant differences between parasitised and non-parasitised A. odoratum plants, but F. novae-zelandiae grown in soil infested with P. projectus produced less dry matter than the non-infested controls (Table 53). The difference was reflected in a greater reduction in foliage dry weight relative to root dry weight. There were no apparent above-ground symptoms in any of the treatments and further, roots of F. novae-zelandiae plants parasitised by P. projectus did not deviate in growth form from roots of plants growing in non-infested

TABLE 51. Dry matter production of A. odoratum seedlings grown in soils infested with P. projectus, or A. neozelandicus, and seedlings grown in non-infested soil.

Treatment		Dry matter production grams oven dry weight			Ratio of root:shoot
		Shoot	Root	Total	
Seedlings grown		0.360	0.302	0.662	0.839
in P. projectus		0.472	0.229	0.701	0.485
infested soil		0.270	0.110	0.499	0.407
		0.205	0.060	0.265	0.293
		0.541	0.203	0.744	0.375
		0.322	0.243	0.565	0.755
		0.630	0.428	1.058	0.679
		0.340	0.150	0.490	0.441
		0.348	0.140	0.488	0.402
		0.388	0.125	0.513	0.370
	Mean	0.388	0.199	0.598	0.505
Seedlings grown in		0.521	0.346	0.867	0.664
A. neozelandicus		0.769	0.478	1.247	0.621
infested soil		0.308	0.161	0.469	0.523
		0.335	0.167	0.502	0.498
		0.721	0.405	1.126	0.562
		0.710	0.395	1.105	0.556
		0.328	0.198	0.526	0.604
		0.520	0.261	0.781	0.502
		0.305	0.195	0.500	0.639
		0.762	0.591	1.353	0.775
	Mean	0.528	0.320	0.848	0.594
Seedlings grown in		0.692	0.396	1.088	0.572
non-infested soil		0.983	0.250	1.233	0.254
		1.074	0.340	1.414	0.318
		0.395	0.209	0.604	0.529
		0.460	0.289	0.749	0.628
		0.175	0.079	0.254	0.451
		0.332	0.130	0.462	0.392
		0.425	0.259	0.684	0.609
		0.391	0.231	0.622	0.591
		0.439	0.202	0.641	0.460
	Mean	0.537	0.238	0.675	0.480

TABLE 52. Dry matter production of F. novae-zelandiae seedlings grown in soil infested with P. projectus, or A. neozelandicus, and seedlings in non-infested soil.

Treatment	Dry matter production grams oven dry weight			Ratio of root:shoot
	Shoot	Root	Total	
Seedlings grown	0.566	0.278	0.844	0.492
in P. projectus	0.472	0.239	0.711	0.506
infested soil	0.270	0.140	0.410	0.518
	0.592	0.261	0.853	0.441
	0.641	0.303	0.944	0.473
	0.422	0.243	0.665	0.576
	0.630	0.428	1.058	0.679
	0.540	0.250	0.790	0.463
	0.388	0.125	0.513	0.322
	0.729	0.370	1.100	0.507
Mean	0.525	0.264	0.789	0.498
Seedlings grown	0.809	0.356	1.165	0.440
in A. neozelandicus	0.713	0.405	1.118	0.568
infested soil	0.561	0.293	0.854	0.522
	0.419	0.275	0.694	0.656
	0.475	0.208	0.683	0.438
	0.695	0.396	1.091	0.570
	0.698	0.448	1.146	0.642
	0.404	0.175	0.579	0.433
	0.709	0.303	1.012	0.427
	0.786	0.392	1.178	0.499
Mean	0.627	0.325	0.952	0.519
Seedlings grown	0.892	0.396	1.288	0.444
in non-infested	0.629	0.262	0.891	0.416
soil	0.519	0.276	0.795	0.532
	0.760	0.389	1.149	0.512
	0.512	0.338	0.840	0.660
	0.629	0.250	0.879	0.397
	0.725	0.349	1.164	0.481
	0.821	0.438	1.259	0.533
	0.731	0.271	1.110	0.371
	0.439	0.252	0.694	0.574
Mean	0.666	0.322	1.006	0.492

TABLE 53: Significance of differences of dry matter production between F. novae-zelandiae seedlings grown in soils infested with P. projectus or A. neozelandicus and seedlings grown in non-infested soil.

Treatment compared	Shoot	Root	Total
P. projectus infested soil	S**	S*	S***
Non-infested soil	W_n =81	W_n =84	W_n =78
A. neozelandicus infested soil	NS	NS	NS
Non-infested soil			

$P(W_n \leq 87) \leq 0.1$*

$P(W_n \leq 82) \leq 0.05$**

$P(W_n \leq 78) \leq 0.025$***

soil, or exhibit evidence of mechanical damage. Rhoades and Lindford (1961), reported that both juveniles and adults of P. projectus and P. dianthus Jenkins and Taylor, 1956, were found within or between cells of the cortex of roots from soil cultures. In the present trial, with the exception of one juvenile found in cortical cells of A. odoratum, P. projectus was not observed in roots of parasitised plants stained with lactophenol cotton blue (Goodey, 1963).

The lack of obvious pathological symptoms other than a general growth reduction of plants parasitised by Paratylenchus species has been noted by previous workers (Faulkner, 1962; Paracer, 1966; Fisher, 1967), and Fisher (1967) observed differences in host susceptibility

to infestations of P. neoamblycephalus Geraert. However in the present case comparisons of susceptibility between A. odoratum and F. novae-zelandiae are invalidated by the fact that the fescue tussock seedlings were transplanted during their early growth. To prevent the dilution of inoculum in infested soil, the amount of soil about the plant roots was reduced to a minimum. In the process mechanical damage to the roots was unavoidable. Root pruning results in a period of stimulated root growth at the expense of top growth (Troughton, 1960). Hence it seems that the effect of P. projectus has been to delay the resumption of shoot growth, or to reduce the amount of concurrent shoot growth during the period of root recovery.

No evidence of post-emergence damping-off was observed.

7.4 EFFECT OF ENVIRONMENTAL VARIATION ON PARASITISED PLANTS

The role of the environment in the epidemiology of plant disease is often more important than the presence of the pathogen itself. The greatest crop damage is usually caused when ecological factors favour the pathogen and interfere with the growth of the host. It was considered that during periods of reduced vitality or of stress imposed by changes in the environment, plants supporting large populations of ecto-parasitic nematodes may be predisposed to the manifestation of symptoms with associated loss of production.

7.4.A Drought

The Broken River area is subject to seasonal environmental extremes with a regular dry period during the summer months (Chapter 6). Plants growing under drought

conditions are subject to water stress and high temperatures. At Broken River, a soil moisture content of 14-15% coinciding with temperatures up to 21°C were recorded in the top 10cm of soil. Taking an approximate critical moisture tension value of 15 bars (Buckman and Brady, 1960) it is apparent that plants growing under these conditions are close to the wilting coefficient (Figure 61). To investigate the role of soil moisture and temperature in disease expression, A. odoratum and F. novae-zelandiae plants parasitised by P. projectus and A. neozelandicus were subjected to conditions approximating the semi-drought summer environment recorded at Broken River.

METHOD

F. novae-zelandiae: Fescue tussock seedlings grown from surface sterilised seed were thinned to three per container eight weeks after sowing. Four weeks later they were inoculated with approximately 100 P. projectus and 50 A. neozelandicus over a period of two weeks. Plants for inoculation were determined by random numbers and all pots received animals within 24 hours of their extraction from the soil. The young tussocks were repotted in 17.5 x 20.0cm containers 16 weeks after inoculation, and when they were thirty-eight weeks old they were transferred to a growth room at 16°C. Plants were acclimatised for four weeks which is sufficient for readjustment to climatic conditions in growth cabinets (Mitchell, 1953). Following the equilibration period, five inoculated pots and five uninoculated pots were randomly sampled for assessment of plant growth and nematode populations. Thirty of the remaining 60 containers were

placed in a second growth room at 21°C. Watering was restricted to induce semi-drought conditions. Both series of plants received the same amount of nutrient solution. At five weekly intervals five inoculated pots and five control pots were randomly sampled for dry matter determinations and nematode population estimates.

A. odoratum: Surface sterilised sweet vernal seed was sown in pots containing approximately 200g of nematode -free soil and soil infested with 110 P. projectus per ml and 14 A. neozelandicus per ml. Three weeks after sowing the plants were thinned to one per pot, and after a further three weeks 10 plants for each treatment were transferred to "stress" conditions of high temperature (21°C) and low moisture, and 10 plants for each treatment were retained under favourable conditions. Dry matter production was determined after four weeks.

RESULTS AND DISCUSSION

Environmental: Environmental data are summarised in Table 54.

F. novae-zelandiae: Dry weights recorded for inoculated plants were less than those of the non-inoculated series after the equilibration period of one month (Table 55), but the differences were not significant. Nematode population estimates based on an aliquot from soil samples from four pots were 98.5 P. projectus per ml and 3.2 A. neozelandicus per ml.

Dry weight determinations of the production of inoculated and non-inoculated plants grown for five weeks under favourable or unfavourable conditions are presented in Table 56. The significance of differences summarised in Table 57 show that the "inoculated unfavourable" plants

TABLE 54. Environmental data recorded for plants maintained under favourable and unfavourable growth conditions

Treatment	Air temperature °C	Soil temperature °C		Soil Moisture %		Humidity %
		Range	X̄	Range	X̄	
F. novae-zelandiae equilibration period	9.0-21.0	10.0-19.5	(16.5)	20-38	(28.0)	83-100
F. novae-zelandiae favourable conditions	14.0-20.5	14.0-19.5	(16.8)	22-35	(27.8)	75-100
F. novae-zelandiae unfavourable conditions	17.0-23.0	19.0-22.5	(20.5)	11-26	(16.5)	81-100
A. odoratum favourable	14.5-20.5	15.0-19.0	(17.4)	17-31	(25.5)	83-100
A. odoratum unfavourable	17.5-22.5	19.0-21.5	(29.9)	11-25	(17.1)	85-100

TABLE 55. Dry matter production if inoculated and non-inoculated F. novae-zelandiae plants after an equilibration period of one month under favourable growth conditions

Treatment		Dry matter production grams oven dry weight			Ratio of root:shoot
		Shoot	Root	Total	
Inoculated plants		26.23	10.98	37.21	0.419
		26.61	14.50	41.11	0.545
		17.74	8.97	26.71	0.506
		24.62	12.79	37.41	0.519
		25.43	15.29	40.72	0.601
	Mean	24.13	12.51	36.63	0.518
Non-inoculated plants		27.66	19.62	47.28	0.709
		36.23	14.30	50.53	0.395
		24.24	11.00	35.24	0.454
		38.82	21.52	60.34	0.554
		19.32	11.21	30.53	0.580
	Mean	29.25	15.53	44.78	0.538

TABLE 56. Productivity of inoculated and uninoculated F. novae-zelandiae plants maintained under favourable and unfavourable growth conditions.

Treatment		Dry matter production oven dry weight grams			Ratio of root:shoot
		Shoot	Root	Total	
Inoculated unfavourable		39.95	17.46	57.41	0.44
		26.33	15.96	42.19	0.61
		32.49	13.84	46.33	0.43
		35.68	14.25	49.93	0.40
		17.14	7.97	25.38	0.46
	Mean	30.37	13.89	44.25	0.47
Uninoculated unfavourable		31.71	22.59	54.30	0.71
		44.44	28.66	71.30	0.64
		34.15	24.59	54.84	0.72
		31.53	23.75	55.28	0.75
		27.52	15.83	43.35	0.57
	Mean	33.87	23.10	56.97	0.68
Inoculated favourable		41.66	23.19	64.85	0.56
		37.61	20.97	58.58	0.56
		41.34	31.43	72.77	0.76
		38.92	26.15	65.07	0.67
		39.63	21.08	60.71	0.53
	Mean	39.83	24.56	64.40	0.61
Uninoculated favourable		50.34	31.62	81.96	0.63
		40.81	23.80	64.61	0.58
		29.90	15.53	45.43	0.52
		42.60	22.15	64.75	0.52
		57.31	32.37	89.68	0.56
	Mean	44.19	25.08	69.29	0.56

produced less root dry matter than either of the control series. Inoculated plants grown under stress produced significantly less dry matter than non-inoculated plants from the favourable environment. However, the differences in total dry matter between parasitised and non-parasitised plants maintained in unfavourable conditions was not significant.

Nematode populations of 146.4 P. projectus per ml and 8.5 A. neozelandicus per ml of soil were recorded from pots maintained under favourable conditions and populations of 74.5 P. projectus from soil in an induced drought condition.

TABLE 57: Significance of differences of dry matter production between parasitised and non-parasitised F. novae-zelandiae plants grown under favourable and unfavourable conditions.

Treatments compared	Root	Shoot	Total
Inoculated unfavourable Control unfavourable	S $W_n = 17$	NS	NS
Inoculated unfavourable Control favourable	NS	NS	NS
Control unfavourable Control favourable	NS	NS	NS
Inoculated unfavourable Control favourable	S $W_n = 17$	S $W_n = 18$	S $W_n = 18$

$P(W_n \leq 19) \leq 0.05$

A. neozelandicus was not recorded in the latter samples, and is not considered to play a significant role in the reduction of dry matter production under the experimental conditions. The differences in final population levels of the two parasites can probably be interpreted in part from the differences in initial levels of inocula, and in part from the differences in generation time between the two species (Chapter 4), but these factors alone seem insufficient to explain the dominance of P. projectus. The evidence supports the suggestion made in Chapter 5, that interspecific competition to the extent of suppression of one species by another may be operative.

At this point in the experiment one of the growth rooms went out of control overnight, precluding the comparison of further samples.

A. odoratum: Analysis of the results (Table 58) summarised in Table 59 revealed a similar pattern of differences for A. odoratum to those obtained with F. novae-zelandiae. Total dry weight of parasitised plants grown under semi-drought conditions was reduced and this was evidenced mainly as a difference between root weights. Non-parasitised plants grown under favourable conditions produced significantly more root and total dry matter than non-parasitised plants grown in a semi-drought environment. There was no apparent difference between nematode-infested plants and nematode-free plants maintained in a favourable environment for growth.

The amount of plant root relative to shoot is of almost constant magnitude for plants of one species under one set of conditions and uniform growth stages (Brouwer, 1966). Variation of environmental conditions alters this ratio until a new balance is achieved. A reduction of soil moisture in the root environment causes

TABLE 58. Productivity of infested and non-infested A. odoratum grown under favourable and unfavourable conditions.

Plant environment		Dry matter production oven dry weight grams			Ratio of root:shoot
		Shoot	Root	Total	
Infested		0.098	0.046	0.144	0.469
unfavourable		0.122	0.048	0.170	0.393
		0.101	0.059	0.160	0.584
		0.068	0.049	0.117	0.721
		0.058	0.041	0.099	0.707
		0.089	0.046	0.135	0.517
		0.132	0.073	0.205	0.553
		0.065	0.032	0.097	0.492
		0.099	0.049	0.148	0.494
		0.063	0.036	0.099	0.571
	Mean	0.089	0.048	0.137	0.550
Non-infested		0.105	0.090	0.195	0.857
unfavourable		0.079	0.069	0.148	0.873
		0.101	0.092	0.193	0.911
		0.106	0.107	0.213	1.009
		0.129	0.139	0.268	1.077
		0.070	0.060	0.130	0.857
		0.131	0.120	0.251	0.916
		0.069	0.051	0.120	0.739
		0.106	0.088	0.194	0.830
		0.075	0.061	0.136	0.813
	Mean	0.097	0.091	0.188	0.888
Infested		0.122	0.095	0.217	0.779
favourable		0.179	0.149	0.328	0.832
		0.054	0.038	0.092	0.704
		0.133	0.093	0.226	0.699
		0.140	0.099	0.239	0.707
		0.145	0.121	0.266	0.834
		0.136	0.078	0.214	0.573
		0.148	0.129	0.277	0.872
		0.168	0.139	0.307	0.827
		0.181	0.129	0.310	0.712
	Mean	0.141	0.107	0.246	0.754
Non-infested		0.100	0.105	0.205	1.050
favourable		0.163	0.126	0.289	0.773
		0.074	0.058	0.132	0.784
		0.163	0.135	0.298	0.828
		0.151	0.102	0.253	0.680
		0.113	0.081	0.194	0.717
		0.189	0.148	0.337	0.783
		0.082	0.053	0.135	0.646
		0.141	0.099	0.240	0.702
		0.143	0.136	0.279	0.951
	Mean	0.122	0.114	0.236	0.707

TABLE 59. Significance of difference of dry matter production between parasitised and non-parasitised A. odoratum plants grown under favourable and unfavourable conditions.

Treatments compared	Root	Shoot	Total
Inoculated unfavourable Control unfavourable	NS	S $W_n = 60$ $P(W_n \leq 65) \leq 0.001$	S $W_n = 79$ $P(W_n \leq 82) \leq 0.05$
Inoculated favourable Control favourable	NS	NS	NS
Control unfavourable Control favourable	S $W_n = 77$ $P(W_n \leq 78) \leq 0.025$	NS	S $W_n = 81$ $P(W_n \leq 82) \leq 0.05$
Inoculated unfavourable Control favourable	S $W_n = 72$ $P(W_n \leq 74) \leq 0.01$	S $W_n = 59$ $P(W_n \leq 65) \leq 0.001$	S $W_n = 69$ $P(W_n \leq 71) \leq 0.005$

reduced shoot growth, whereas root growth is relatively insensitive (Brouwer, 1966). Comparison of the root: shoot ratios for the control treatments in each experiment (Tables 56 and 58), indicates that there has been a relative increase of root to shoot in non-inoculated plants grown in semi-drought conditions. Increased temperature generally decreases the root:shoot ratio (Brouwer, 1966; Davidson, 1969). Hence it would appear that moisture was the main factor influencing growth of control plants under the experimental conditions.

P. projectus has been shown to be capable of long survival in the non-feeding pre-adult juvenile stage (Rhoades and Lindford, 1959; 1961a). Further, Rhoades and Lindford (1961a), reported that all stages of the nematode are tolerant to moisture percentages below half that of the wilting point. Under the moisture conditions of the present experiments, feeding probably continued, although activity may have been reduced.

7.4.B Recoverability of A. odoratum

Transferring plants from a favourable environment to semi-drought conditions altered the root:shoot ratio. With the resumption of favourable conditions a root:shoot balance approximating that of plants grown in the favourable environment throughout should be achieved (Brouwer, 1963; 1963b; 1966).

The normal growth response to environmental change was altered in drought stressed plants by the presence of ecto-parasitic nematodes in the rhizosphere. An experiment was carried out to investigate the effects of root parasitism on the subsequent production of plants returned to favourable growth conditions.

Because of the apparent lack of pathogenicity exhibited by A. neozelandicus in the preceding experiments, the low

population levels established, and the lack of persistence of these populations under adverse conditions, inoculations were confined to P. projectus.

METHOD

Single A. odoratum seedlings were established under favourable growth conditions in pots containing soil infested with 95 P. projectus per ml of soil and pots containing non-infested soil. Six weeks after sowing five nematode infested plants and five control plants were subjected to moisture stress at 21°C for four weeks. At the end of this period they were returned to favourable conditions, and harvested five weeks later. A similar series of infested and non-infested plants, maintained under favourable moisture conditions in the 16°C growth room were harvested at the same time.

RESULTS AND DISCUSSION

The results of dry matter determinations are presented in Table 60: the significance of differences are summarised in Table 61.

There are no significant differences between parasitised and non-parasitised plants which received the same treatment throughout the experiments, but those plants which were subjected to a period of stress produced significantly less dry matter of roots and shoots than plants maintained under favourable conditions.

When a limiting factor to plant growth such as water is restored, accumulated carbohydrates, and carbohydrates produced by current photosynthesis are mainly used for the development of the shoot (Brouwer, 1966).

TABLE 60. Dry matter production of infested and non-infested A. odoratum plants maintained under favourable or unfavourable growth conditions for a period and then transferred to favourable conditions for a further period.

Treatment	Dry matter production grams oven dry weight			Ratio of root:shoot
	Shoot	Root	Total	
Infested	0.550	0.435	0.985	0.7909
unfavourable	0.382	0.389	0.771	1.018
	0.310	0.342	0.652	1.103
	0.223	0.242	0.465	1.085
	0.449	0.417	0.866	0.929
Mean	0.383	0.365	0.749	0.985
Non-infested	0.375	0.373	0.749	0.992
unfavourable	0.439	0.565	1.004	1.287
	0.449	0.443	0.892	0.987
	0.341	0.329	0.670	0.965
	0.408	0.410	0.818	1.005
Mean	0.403	0.424	0.827	1.047
Infested	0.621	0.509	1.120	0.833
favourable	0.695	0.498	1.193	0.716
	0.649	0.617	1.266	0.951
	1.010	0.931	1.941	0.922
	1.141	1.111	2.252	0.974
Mean	0.821	0.733	1.554	0.879
Non-infested	0.959	1.087	2.046	0.882
favourable	1.114	1.020	2.134	1.092
	0.601	0.622	1.223	0.966
	0.572	0.585	1.157	0.978
	1.119	1.101	2.220	1.016
Mean	0.873	0.883	1.756	0.987

TABLE 61: The significance of differences of dry matter production between parasitised and non-parasitised plants grown under stress and then returned to favourable conditions.

Treatments compared	Root	Shoot	Total
Inoculated stress Control stress	NS	NS	NS
Inoculated favourable Control favourable	NS	NS	NS
Inoculated stress Control favourable	S $W_n = 15$	S $W_n = 15$	S $W_n = 15$
Control stress Control favourable	S $W_n = 15$	S $W_n = 15$	S $W_n = 15$

$$P(W_n \leq 15) \leq 0.005$$

This continues until the absorption capacity of the root system becomes limiting. At such a time, shoot growth rate decreased and more carbohydrates become available for root growth. An equilibrium appropriate to the new situation has then been established. Comparison of the root: shoot ratios (Table 60) indicates that this situation has been approached. Deviations from the normal growth pattern induced by nematode parasitism of drought stressed plants do not appear to persist when plants are restored to an environment more conducive to plant growth.

7.4.C Defoliation of A. odoratum

Clipping of forage legumes has been associated with increased severity of infection by root-rotting fungi (O'Rourke and Millar, 1966; Siddiqui, Halisky and Lund, 1968) and predisposition to attack by weak pathogens (Fulton and Hansen, 1960). Pasture plants at Broken River are subject to periodic grazing. An experiment was devised to investigate the effects of root parasitism by P. projectus on subsequent production of partially defoliated A. odoratum plants.

METHOD

Eleven A. odoratum seedlings were established singly in pots containing soil infested with 95 P. projectus per ml. A second series of 11 seedlings was established in non-infested soil. The plants were maintained in the 16°C growth room, under favourable moisture conditions. Application of nutrient was discontinued after six weeks. Nine weeks after sowing the foliage was removed from each plant by cutting to 2.5cm from the soil level, and the dry weight was determined. At weekly intervals for three weeks there-after the foliage was removed at the same level and the shoot dry weight recorded. On completing the final clipping, the dry weights of stem and root were ascertained.

RESULTS AND DISCUSSION

The significance of differences of dry matter production between treatments (Table 62) are summarised in Table 63.

A reduction in the assimilatory surface of a plant by cutting or grazing causes a marked reduction in root growth (Jacques and Schwass, 1956; Baker,H.K. 1957: Troughton, 1957; del Pozo, 1963) and a reduction in nutrient uptake (Ostwalt, Bertrand and Teel, 1959; Davidson and Milthorpe, 1965).

TABLE 62. Dry matter production of defoliated A. odoratum plants growing in soil infested with P. projectus and nematode-free soil.

Treatment		Dry matter production in grams								Root:shoot
		Under shoot				Stem	Total foliage	Root	Total plant	
		First harvest	First regrowth	Secondary growth	Third regrowth					
		0.132	0.059	0.031	0.012	0.069	0.303	0.072	0.375	0.238
		0.183	0.057	0.057	0.038	0.044	0.379	0.141	0.520	0.372
		0.095	0.052	0.023	0.014	0.049	0.181	0.079	0.260	0.436
		0.172	0.060	0.043	0.038	0.088	0.401	0.137	0.538	0.342
Infested		0.080	0.060	0.020	0.017	0.041	0.218	0.069	0.287	0.316
soil		0.156	0.025	0.020	0.017	0.047	0.265	0.078	0.343	0.294
		0.149	0.060	0.030	0.033	0.063	0.335	0.113	0.448	0.337
		0.063	0.090	0.018	0.022	0.021	0.214	0.089	0.303	0.416
		0.060	0.031	0.020	0.009	0.019	0.139	0.030	0.169	0.212
		0.095	0.041	0.040	0.032	0.058	0.266	0.118	0.384	0.444
		0.121	0.052	0.036	0.027	0.063	0.299	0.091	0.390	0.304
	Mean	0.1187	0.0534	0.0307	0.0235	0.0511	0.2718	0.0924	0.3652	0.337
		0.094	0.040	0.028	0.024	0.032	0.218	0.078	0.296	0.358
		0.131	0.091	0.046	0.042	0.083	0.393	0.146	0.539	0.371
		0.119	0.052	0.020	0.028	0.031	0.250	0.112	0.362	0.448
Non-infested		0.192	0.110	0.029	0.043	0.097	0.471	0.160	0.631	0.340
soil		0.091	0.045	0.031	0.019	0.026	0.212	0.079	0.291	0.373
		0.149	0.073	0.042	0.040	0.051	0.335	0.150	0.485	0.448
		0.151	0.091	0.041	0.047	0.058	0.388	0.160	0.498	0.412
		0.237	0.061	0.028	0.022	0.046	0.394	0.104	0.498	0.264
		0.141	0.078	0.044	0.029	0.063	0.355	0.113	0.468	0.318
		0.199	0.059	0.042	0.050	0.088	0.398	0.146	0.544	0.367
		0.132	0.055	0.031	0.021	0.070	0.319	0.067	0.386	0.210
	Mean	0.1487	0.0695	0.0382	0.0332	0.0550	0.3394	0.1195	0.454	0.355

TABLE 63: Significance of differences of dry matter production of defoliated A. odoratum plants growing in soil infested with P. projectus and nematode-free soil

Treatment compared	First Harvest	First Regrowth	Second Regrowth	Third Regrowth
Parasitised plants Non-parasitised plants	NS	S* $W_n = 104$	NS	S** $W_n = 97$

Treatment compared	Stem	Total Shoot	Root	Total plant
Parsitised plants Non-parasitised plants	NS	S* $W_n = 101$	S* $W_n = 102$	S** $W_n = 100$

$P(W_n \leq 106) \leq 0.1$ *

$P(W_n \leq 100) \leq 0.05$**

There is an immediate decrease in concentration of carbohydrate accumulated in roots and other storage organs as sugars, fructosans and starch (Sullivan and Sprague, 1953; Davidson and Milthorpe, 1965). Troughton (1957) considered that this was due to transference of material to the remaining shoot tissue for utilisation in new growth. Root activity does not resume until regrowth of shoot establishes a root:shoot ratio approximating that which existed prior to cutting (Brouwer, 1963a; del Pozo, 1963, Davidson and Milthorpe, 1965).

The difference in total plant dry matter (Table 63) represented a slight reduction in root and shoot dry matter production by nematode infested plants. Shoot production by parasitised plants was significantly reduced at the first and third regrowth harvests (Table 63), but the differences between treatments at the second harvest were not significant. Whilst parasitised plants produced less dry matter, the difference at the first harvest, although not statistically significant, indicates that the effects of nematode parasitism may have been manifest before the plants were defoliated (Figure 62). Defoliation could have emphasised initial differences, but in view of the results the treatment can not be regarded as a major predisposing factor under the experimental conditions. The gradual 'reduction' in regrowth after successive harvests suggests the depletion of reserve materials available for foliage regeneration.

Shoot pruning has been shown to cause an increase in some populations of root parasitic nematodes (Dolliver, 1961; MacDonald and Mai, 1963; MacDonald 1966). The inoculum of P. projectus was reduced from 95 per ml to 38.5 per ml at the termination of the present trial. With the relatively small amount of root available for parasitism during the first few weeks of seedling growth this can probably be interpreted in terms of high initial nematode mortality.

7.5 LABORATORY TRIALS

The constancy of the root:shoot ratio for any one plant species at the same growth stage indicates that it is precisely regulated (Brouwer, 1966). In drought stressed plants parasitised by P. projectus the pattern of growth was altered, which suggests that the growth regulatory mechanism may have been affected.

Since Goodey (1948) first suggested that plant growth substances may be involved in nematode induced plant galls, several authors have implicated auxin-type compounds in plant-nematode interrelationships (e.g. Krusberg, 1963; Dropkin, 1969). Dropkin (1969) cited reports of auxin activity and auxin inactivators in nematode extracts and changes in the levels of endogenous growth regulators in the presence of nematode infections. In his view the "evidence for nematode induced changes in plant growth regulatory substances is fairly strong". Most of the examples in the literature however, refer to the host-parasite relationship of endo-parasitic nematodes which incite hyperplastic symptoms (Balasubramanian and Rangaswami, 1962; Bird, 1962; Yu and Viglierchio, 1964; Viglierchio and Yu, 1965, 1968; Sandstedt and Schuster, 1966; Cutler and Krusberg, 1968; Setty and Wheeler, 1968). Evidence of pathogenesis involving plant growth regulators produced by ecto-parasitic nematodes is not well documented, but the induction of changes in root morphology which could be linked with responses to growth regulatory substances have been demonstrated for some species (Rhode and Jenkins, 1957; Zuckerman, 1961; Van Gundy, 1961, Seinhorst, 1966a; Deubert, Norgren, Paracer and Zuckerman, 1967; McElroy and Van Gundy, 1967; Paracer, Waseem and Zuckerman, 1967).

Laboratory experiments of a preliminary nature were carried out to investigate the host response to parasitism by P. projectus and A. neozelandicus.

7.5.A Root elongation

METHOD

Short rotation ryegrass was shown to be a suitable

host for both nematode species (Chapter 4). Because of its rapid germination and growth responses, surface sterilised ryegrass seed was used in seedling growth experiments.

Seedlings were germinated in Petri plates containing 1.0% water agar. The dishes were placed at an angle of about 45° to promote growth in one direction. In consecutive experiments seedlings were inoculated with 100 surface sterilised P. projectus or 100 surface sterilised A. neozelandicus when the emerging radicle was about 3.0mm long. The rate of root elongation was measured at 24 hour intervals over a period of 72 hours for nematode parasitised seedlings and for nematode-free seedlings.

RESULTS AND DISCUSSION

There were no significant differences in the rate of seedling root elongation between treatments (Table 64).

According to the classical view, auxin content of intact roots is well above the optimal level, and normally application of auxin to roots causes an inhibition of elongation (Larsen , 1951; Aberg, 1957). Inhibition does not result from a general decrease in the intensity of the growth processes, but rather from a change in their course (Aberg, 1957). This is demonstrated by the characteristic sub-apical swelling of inhibited roots which has been described by several workers (e.g. Cholodney, 1931; Sun, 1956). No "stubby-root" symptoms were observed in the present trial.

7.5.B Root geotropism

The interpretation of auxin action in root geotropism is still confused (Audas, 1969). Recent work

TABLE 64: Rate of root elongation of nematode parasitised and non-parasitised ryegrass seedlings grown in water agar.

Treatment	Increments of root growth (mm) recorded at 24 hour intervals			Mean growth increment (mm) per 24 hours	Total growth increment (mm) for 72 hours
	1	2	3		
P. projectus inoculated seedlings	5.2	5.5	6.8	5.8	17.5
	7.5	9.0	11.2	9.2	27.7
	15.0	17.2	23.2	18.5	55.4
	7.2	6.0	8.2	7.1	21.4
	5.0	4.7	4.8	5.0	15.0
A. neoscelandicus inoculated seedlings	7.0	7.2	9.2	7.8	23.4
	5.2	7.5	7.6	6.8	22.1
	8.5	8.5	8.7	8.6	25.7
	9.0	8.2	8.7	8.6	27.9
	17.5	13.5	12.5	14.5	43.5
Uninoculated seedlings	8.5	9.0	9.0	8.8	26.5
	7.5	7.0	7.0	7.2	21.5
	10.2	9.5	12.5	10.7	32.2
	5.0	5.5	4.5	5.0	15.3
	9.7	10.5	7.0	9.1	27.2

(Chadwick and Burg, 1970) implicates differential cell elongation induced by IAA dependent ethylene production. As the action of auxin is rapid (Thimann, 1969), it was considered that the possible production of auxins, auxin-like compounds, or auxin inhibitors by P. projectus or A. neozelandicus during feeding may be evidenced by an effect on the geotropic response of seedling roots.

METHOD

i) Ryegrass seedlings were germinated in 7.5cm tubes containing 1.0% water agar. When the radicle was approximately 3.0-5.0mm long the seedlings were inoculated with 100 surface sterilised P. projectus or 100 A. neozelandicus.

ii) Seedlings were germinated in Petri dishes containing 1.0% water agar and inoculated as above. The direction of root growth was controlled by incubating the plates at an angle of 45^{o}.

When the seedling roots were about 2.5cm long, the containers were re-orientated so that the roots were horizontally positioned. Each experiment was replicated six times, and for each treatment non-parasitised seedlings were examined at the same time.

RESULTS AND DISCUSSION

The geotropic response was well evident after 48 hours. With the exception of one P. projectus replicate and one control treatment, all seedlings exhibited normal geotropism.

7.5.C Cell permeability

Altered cell permeability appears to be characteristic of many diseased plant tissues (Wheeler and Hanchney, 1968). Cells in contact with a pathogenic agent may become "leaky" and show a loss of ability to accumulate salts and other materials. Such an effect could contribute to reduced dry matter production under conditions of high soil moisture tension.

METHOD

Surface sterilised ryegrass seedlings and white clover seedlings were germinated in Petri dishes containing 1.0% water agar. The seedlings were inoculated with 100 surface sterilised P. projectus or 100 A. neozelandicus, and when the roots were about 1.5cm long the dishes were inverted to promote growth toward the surface of the agar until the tip emerged by about 6mm. They were then suspended in a solution of rhodamine B (Weischer, 1957) and the translocation of the dye observed under a compound microscope.

RESULTS AND DISCUSSION

There was no apparent difference in dye uptake in roots parasitised by either pathogen, or in non-parasitised roots.

Weischer (1957), found that cells of carrot root infected by Pratylenchus exhibited altered cells permeability. By suspending infected roots in a dilute solution of rhodamine B he demonstrated that dye concentrated in the tissues around the feeding nematodes. Cells parasitized by Paratylenchus were unchanged, from

which he suggested that Pratylenchus effects the permeability of tissues for some distance from the feeding site, and that feeding of Paratylenchus on the root epidermis does not. Thus the evidence indicates that ecto-parasitic Paratylenchus and Aglenchus do not alter cell permeability during feeding.

7.6 DISCUSSION

Root parasitism of F. novae-zelandiae and A. odoratum by A. neozelandicus caused no reduction in dry matter production under the experimental conditions. However, levels of inoculum were not as high as those sometimes recorded in the field (see Chapters 5 and 6), and although the evidence indicates that large losses of production are unlikely to be caused by this parasite, the possibility that some damage may be incurred can not be ruled out. Parasitism of F. novae-zelandiae seedlings by P. projectus was associated with a significant reduction of total dry matter in an environment favourable to plant growth, but the results were not consistent over the series of experiments even with inoculum loads up to twice the maximum level recorded from field samples. Under conditions favouring plant growth P. projectus must be regarded as a weak pathogen. But P. projectus parasitised plants grown under stress imposed by a semi-drought environment deviated from the normal growth response with subsequent loss of dry matter production. Both plants and nematodes respond to fluctuations in moisture, temperature and other gradients, but their sensitivity to change, and the nature of their responses are not the same. A change of environmental circumstances may therefore result in a short term or long term shift in the balance of a host-parasite relationship. The introduction

of moisture stress in the present experiments caused such a shift. It seems that P. projectus induces changes in the host which disrupt the normal growth response mechanism, or which render the plants more susceptible to stress; or that physiological change in the host caused by environmental extremes result in a situation in which the plant is less tolerant of feeding by the nematode, or both. Hence at any one instant, the effect of a particular density of P. projectus feeding on plant roots under field conditions may be a function of the prevailing environment.

The mechanisms invoked in considerations of the host-parasite relationship of phytophagous nematodes are various (see reviews by Dropkin, 1955; Christie and Perry, 1959; Mountain, 1960; Seinhorst, 1960; Krusberg, 1963; Dropkin, 1969). Elucidation of the processes operative in the present trials was not possible from the data obtained, but the implications of the results warrant discussion.

A. neozelandicus and P. projectus produce a secretion during feeding (Chapter 4). A dome of granular material forms around the orifice of the protruding stylet while cytoplasmic streaming continues within the parasitised cell. Feeding does not cause death of the cell. Evidence suggesting changes in the physiology of nematode parasitised plants have been reported for both genera: Deubert et al (1967), showed that feeding of T. emarginatus on corn roots under gnotobiotic root conditions, resulted in an increase of the diameter of cell nuclei in the vicinity of the feeding nematode, and Coursen and Jenkins (1958), attributed increased tillering in tall fescue to infestation by P. projectus. These observations indicate that plant growth processes may be altered without evidence of physical damage or gross morphological changes to the parasitised roots. Recent work demonstrating

changes in plant physiology associated with nematode feeding (Faulkner and Bolander, 1969; Faulkner, Bolander and Skotland, 1970) support this view. Faulkner et al (1970), found that the presence of Pratylenchus minyus Sher and Allen 1953, increased the incidence and severity of peppermint wilt incited by Verticillium dahliae even when the interacting organisms were isolated on separate root systems of the same plant.

Many of the symptoms produced by nematode parasitised plants are analogous to the effects of auxin imbalance, and there are several reports of increases or decreases in the concentration of these substances in the presence of nematode infection (Dropkin, 1969). Viglierchio and Yu (1968) showed that the kind of auxin attributed to the presence of a particular nematode appeared to be superimposed on that normally present in the host. Although most of the work carried out has involved endo-parasites, the nature of the host response to feeding of some ecto-parasitic forms suggests the involvement of growth regulatory substances. For example, Zuckerman (1961), reported that cells of cranberry "contiguous to the point of penetration" of Hemicycloiphora similis Thorne 1955, stopped elongating while normal growth of cells on the opposite side caused a distinct curvature. Recently, Kirk and Jacobs (1970) reported acropetal polar movement of IAA-^{14}C in roots. If these results with isolated sections are applicable to the intact plant, then auxin movement down the root axis to the root tip is indicated, and feeding of nematodes along the length of the root may have added significance in effects on the sub-apical regions of cell elongation.

Nematodes have been associated with plant injury caused by reduced uptake and translocation of nutrients

and/or water (Dropkin and King, 1956; Hunter, 1958; Van Gundy and Martin, 1961; Oteifa and Elgindi, 1962; Shafiee and Jenkins, 1963; Heald and Jenkins, 1963; O'Bannon and Reynolds 1965; Jenkins and Malek, 1966). O'Bannon and Reynolds (1965) reported that in root-knot nematode infected plants sufficient water for growth moves to the upper portions of the plant even when roots are severely galled, but when the soil moisture dropped below field capacity growth was reduced. Much of the damage can probably be attributed to disruption of the vascular system in a manner similar to that of some of the classical vascular wilt pathogens (e.g. Wood, 1967). However, "vascular wilt organisms" may disturb the functioning of the whole plant beyond the immediate area of cell damage in the absence of mechanical blockage induced by abnormal plant growths. In these cases quite small quantities of toxins, growth regulating substances or high molecular weight compounds may have marked systemic effects on the host (Wood, 1967). The existence of similar effects in plants parasitised by ecto-parasitic nematodes is an interesting possibility.

Physiological changes take place in plants subjected to drought conditions (Vaadia, Raney and Hagan, 1961; Henckel, 1964). Water stress may induce changes in viscosity and permeability of protoplasm (Subramanian and Saraswathi-Devi, 1959); and changes in the sub-microscopic structure of cells (Nir, Klein and Poljakoff-Mayber, 1969; Nir, Poljakoff-Mayber and Klein, 1970). Physiological modifications of such a nature may affect the relationships between plants and phytopathogens and it seems likely that the tolerance of the host to nematode parasitism may be reduced.

The difficulty involved in controlling the soil environment and standardising plant growth is indicated by

the variability observed in root:shoot ratios between plants within treatments. This variation could mask small differences between treatments and may disguise the significance of real treatment effects. Ideally, successive measurements on a series of individual plants would be taken, but repeated measurements are impossible for plants grown in soil and samples must be taken from a population. Troughton (1968) has shown that considerable variation may occur between plants within a variety: for example, the greater a genotype's shoot growth, the greater its root growth but the smaller its root relative to shoot growth. To minimise genotypic variability, it could be advisable in future initial investigations of nematode plant interactions to take samples from populations consisting of plants originating from the same parent plant. It is evident from the experiments carried out on plants subjected to semi-drought conditions, that to detect and interpret small differences between treatments in total productivity studies, it is necessary to split growth into its component parts. Troughton (1968), listed six plant characters from which six ratios of growth rates which he considered to be morphologically meaningful could be measured (viz. root relative to shoot growth, number of roots per tiller, tiller size, root size, main root length, weight per unit length of main root). These measurements enable the detection of small differences with accuracy, and enable the separation of differences due to size and other causes (Troughton, 1968). Hence more detailed consideration of growth parameters may be invaluable to plant pathologists in situations in which large populations of plants are impractical and strict environmental control is not possible.

8. CONCLUDING REVIEW

Recent trends in nematode ecology and plant nematology empasise the need for integrated studies in soil ecology. Too often the evaluation of individual components of the soil ecosystem does not facilitate the description and understanding of the complex processes at work. Ecological studies of soil nematodes are complicated by the biological diversity of the Nematoda and the heterogeneity of the soil ecosystem. By definition, such studies require the integration of many disciplines, including the plant sciences, microbiology, and the soil sciences. In applying the 'holistic' approach to the present study, the interdependence of these philosophies has been demonstrated.

During the initial stages of the study, sampling and extraction procedures were evaluated and standardised. In the course of this work, the nematode fauna was characterised and an indication of the relative abundance of species obtained. A modification of Seinhorst's (1956, 1962) elutriation method was chosen for extraction of nematodes from soil samples. Samples of 37.5ml yielded the greatest number of nematodes using Seinhorst's technique. Because of the time-lag between collection of samples and their extraction, the effect of the duration of cool storage on the number of nematodes harvested was examined. To assess the reliability of extraction techniques an apparatus consisting of a cylinder containing perforated dispersion baffles was developed which enabled aliquots to be drawn from bulked samples. Extraction efficiency varied between species (e.g. 46.7% for *W. otophorum*, and 90.5% for *A. neozelandicus*); most losses were considered to occur in the sieving processes. Sixty-nine species of

nematodes were recorded on more than one occasion from 50 samples extracted during preliminary analyses.

Knowledge of the feeding habits of many species of nematodes is limited, indeed the feeding habits of some groups are unknown. Therefore, as a pre-requisite to considering the ecology of nematodes at Broken River, experiments were carried out to identify the food requirements of the common species. The nematodes were considered in two groups, non-stylet-bearing species which were cultured on bacteria, and stylet-bearing species which, under culture conditions, fed on one or more of the following food types: seedlings; plant callus tissue; fungi; moss protonemata; nematodes and other soil animals. Populations of 16 non-stylet-bearing species were established under xenic conditions, 11 of which were cultured in a monoxenic condition. Twenty-three species of stylet-bearing nematodes were cultured. The degree of food specialisation varied between species; some were obligate to one food type, others fed on a range of foods, sometimes including both animal and plant tissue. The feeding habits of some species had not previously been recorded.

To develop an understanding of interrelationships between nematodes and various components of the soil fauna and flora, nine species of nematodes representing a range of feeding types and taxonomic groups were selected for detailed biological studies. Emphasis was placed on life-histories, host ranges and host-parasite relations. Where necessary, the systematics of the nematodes were discussed. In addition, investigations into the frequency of occurrence and host-parasite relationships of nematode predacious fungi were carried out.

Because of the confusion regarding the taxonomic status of species and sub-genera of Tylenchus, the systematics

of Tylenchus species from Broken River were considered in some detail. Cephalenchus was raised to generic rank, and Aglenchus was accepted as a valid genus. A new species of Cephalenchus and two species of Tylenchus were described. A. neozelandicus n. comb. was transferred from Tylenchus to Aglenchus, and the original description ammended.

The species considered in biological studies with key words referring to the major aspects considered were as follows:

i) Paratylenchus projectus, penetration and feeding, generation time, host range;

ii) Aglenchus neozelandicus, host range, penetration and feeding, life-history, attraction to hosts, host selection;

iii) Tylenchus rikus n. sp. feeding, host range, general life-history, gonad development, moulting, laying behaviour;

iv) Aphelenchus bicaudatus, morphological variabilit y, penetration and feeding, host suitability, embryonic development, effect of temperature on egg hatching and population development, host attraction and host selection;

v) Seinura demani, host range and feeding, prehatch and post hatch development, mating behaviour, sex attraction;

vi) Deladenus durus, morphology, feeding and host range, copulation, egg development, egg laying, post-embryonic development;

vii) Aporcelaimellus paraamylovorus n. sp., descriptions, host range, penetration and feeding, egg hatching, post-embryonic development, moulting, defaecation;

viii) Tylencholaimus sp., morphology, host range,

penetration and feeding, defaecation, embryonic development, post-embryonic development;

ix) Acrobeloides sp., generation time, effect of temperature on hatching, host range;

x) Nematode-trapping fungi, frequency of occurrence of species isolated, description of Harposporium tarum n. sp., disease cycle, morphology and notes on other species isolated.

Departures from random distributions present problems in the quantitative estimation of nematode populations and in the interpretation of observed variation. Hence the distribution of nematodes at Broken River was investigated in detail, and the number samples required to detect seasonal fluctuations with reasonable accuracy was determined. In an attempt to minimise the problems inherent in aggregated distributions, species were assigned to functionally related groups on the basis of feeding habits. Analyses were carried out for ecological feeding groups and for some of the common species in the fauna.

Distribution patterns are closely related to the extent of the area sampled, and to the homogeneity within that area. Two patterns of sampling were therefore adopted. In the first, a series of closely spaced samples was taken from improved pasture and, about 200 metres distant, a similar series was taken from unimproved grassland. In the second, stratification was included in samples from plots of about 400 square metres on the two sites, and in samples confined to improved tussock grassland. Stratification was based on the fescue tussock rhizosphere as the sample environment because of the dominant position of the tussocks in the community, and because the tussocks cover a relatively large ground area thereby ensuring

a standard reference unit.

The distribution of nematodes in fescue tussock grassland was shown to be contagious. Aggregation of total numbers was superimposed upon aggregation of ecological feeding groups and species. The implication of aggregation on the number of samples required for valid population estimates was emphasised, and the biological significance of aggregation was discussed. The greatest density of nematodes occurred in the top 10-15cm of soil, with maxima near the surface.

The composition of the nematode fauna of improved and unimproved tussock grassland was compared. Although there was little change in the species composition of the fauna on both sites, pasture improvement significantly increased the density of the nematode population. The increase was due mainly to an increase in the numbers of higher plant feeding nematodes.

Fluctuations in nematode numbers and environmental parameters were recorded from soil samples taken at intervals during December 1968 and February, 1970. Two patterns of sampling were carried out:

i) Nematodes were extracted from a series of samples taken to a standard depth in the fescue tussock rhizosphere to obtain an estimate of seasonal fluctuations in the horizontal plane;

ii) Samples were taken at a series of levels in the tussock and inter-tussock rhizosphere to estimate variation in the vertical plane.

Interpretation of fluctuations was complicated by the aggregated distributions of nematodes within the fauna, and by the presence of a lag phase between changes of the environment and changes of nematode numbers. By taking large numbers of samples and by including specific populations in ecological feeding groups, distribution problems were

minimised. By adjusting nematode numbers relative to environmental data, compensation for the lag phase was made. Despite these manipulations, the proportion of explained variation in step-wise multiple regressions of environmental factors with nematode numbers did not exceed 50%. The sources of variation are complex. In discussion throughout Chapter 6, it has been clearly shown that interpretation of trends depends on some understanding of the biology of the main species comprising the nematode fauna.

Numbers of nematodes per square metre in the top 10cm of soil under fescue tussock plants fluctuated from 7.7millions to 10.6millions over a full years cycle. Minimum numbers occurred in winter, rising in late spring with late summer maxima and a decline during autumn. A similar trend was shown in the inter-tussock zone, but fluctuations involving greater than two-fold increases in numbers were observed.

Vegetation was shown to be important in quantitative effects on seasonal trends of nematodes. Whereas a single late summer peak was recorded for nematodes from tussock samples, a small second peak during spring, occurred in the inter-tussock zone. A buffering action of tussocks on fluctuations of environmental factors, and differential plant growth patterns were implicated.

Temperature was considered to be the most limiting factor to the development of the nematode fauna at Broken River. Temperature was critical during autumn, winter and spring, and its effects are manifest through a direct action on nematodes, through limiting population replacement, and through associated effects on the soil fauna and flora. Soil moisture was probably most limiting to nematodes and to the availability of food in the top soil during summer months.

Ectoparasitic higher plant feeding nematodes were shown to be prevalent in soil at Broken River. Experiments were carried out to assess the effect of feeding of A. neozelandicus and P. projectus on growth of F. novae-zelandiae and A. odoratum.

Root parasitism by A. neozelandicus caused no reduction in dry matter production. Under conditions favouring plant growth, and at inocultum loads up to twice the maximum level recorded from field samples, P. projectus was found to be a weak pathogen. But P. projectus parasitised plants grown under stress imposed by a semi-drought environment deviated from the normal growth response of root and shoot growth with subsequent loss of dry matter production. The deviations did not persist when plants were restored to an environment more conducive to plant growth. No significant differences were recorded between parasitised and non-parasitised plants in which the assimilatory surface was reduced by cutting.

The evidence indicated that growth regulatory factors may have been involved in the plant response. However, nematode feeding did not affect the rate of elongation or the geotropic response of parasitised seedling roots grown in agar, nor was the permeability of cells about the feeding site altered.

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APPENDIX I. Permanent pasture seeds mix overdrilled into the sampling area in spring of 1954.

	lb
Perennial ryegrass	15
Short rotation ryegrass	5
Cocksfoot	5
Tall oat grass	5
Timothy	3
Montgomery red clover	1
Alsike	1
White clover	2
Sheeps burnett	3
Total	40

Applied at half rates (i.e. 20lb/acre)

APPENDIX II. Fertilizer applications between 1954 and 1964.

45cwt lime per acre per annum 1954-1957,
17½cwt super phosphate (equivalent) per acre per annum 1954-1964.

APPENDIX III. Recipe for pea-agar.

10oz macerated frozen peas; make up to a litre with distilled water,
5g sucrose,
15-20g agar,
steam for 30 minutes, filter through cheese-cloth, autoclave 15 minutes at 15lb.

APPENDIX IV. Nematodes extracted from samples taken at 15cm spacings along a transect between two fescue tussock (F. novae-zelandiae) plants.

A. Improved tussock grassland.

Sample 1.

Plant rhizosphere sampled: Festuca novae-zelandiae.

Species	%	No. per ml soil
Paratylenchus projectus	26	34.15
Aglenchus neozelandicus	2	2.63
Tylenchus maiakus	36	47.28
Aphelenchus avenae	1	1.31
Dorylaimellus sp.	7	9.19
Aporcelaimellus paraamylovorous	5	6.57
Aporcelaimus species 2	4	5.15
Eudorylaimus sp.	5	6.57
Mondchus propapillatus	2	2.63
Plectus sp. (P. inquirenda type).	1	1.31
Plectus sp. (P. armatus type).	1	1.31
Chromodora sp.	2	2.63
Acrobeloides sp.	2	2.63
Eucephalobus sp.	1	1.31
Cervidellis species 2.	1	1.31
Genus et sp. indent.	1	1.31
Miscellaneous	3	3.94
	Total	131.33

APPENDIX IV continued.

A. Improved tussock grassland.

Sample 2.

Plant rhizosphere sampled: Anthoxanthum odoratum.

Species	%	No. per ml soil
Paratylenchus projectus	29	29.43
Tylenchus maiakus	4	4.06
Tylenchus species 2.	1	1.01
Dorylaimellus sp.	2	2.03
Aporcelaimus species 1.	3	3.04
Dorylaimus sp.	1	1.01
Tylencholaimellus montanus	1	1.01
Tylencholaimus sp.	2	2.03
Aporcelaimus species 3.	4	4.06
Dorylaimus species 1.	3	3.04
Labronema sp.	10	10.15
Nygolaimus (Paravulvus) sp.	9	9.13
Dorylaim indent.	6	6.08
Mesorhabditis sp.	3	3.04
Acrobeloides sp.	2	2.03
Eucephalobus sp.	3	3.04
Cervidellis species 1.	5	5.07
Wilsonema otophorum	1	1.01
Plectus sp. (P. parietinus type)	2	2.03
Prismatolaimus sp.	1	1.01
Genus et sp. indent.	2	2.03
Miscellaneous	6	6.08
	Total	101.42

APPENDIX IV continued.

A. Improved tussock grassland.

Sample 3.

Plant rhizosphere sampled: *Cerastium holosteroides*.

Species	%	No. per ml soil
Paratylenchus projectus	30	37.96
Tylenchus maiakus	4	5.06
Tylenchus species 1.	2	2.53
Cephalenchus tahus	4	5.06
Aphelenchus bicaudatus	6	7.59
Aphelenchus avenae	1	1.27
Seinura demani	6	7.59
Dorylaimellus sp.	16	20.24
Aporcelaimus species 1.	5	6.33
Dorylaimus species 2.	1	1.27
Aporcelaimus species 3.	1	1.27
Nygolaimus (*Paravulvus*) sp.	4	5.06
Cervidellis species 2.	1	1.27
Mesorhabditis species 2.	1	1.27
Acrobeloides sp.	11	13.92
Monhystera sp.	1	1.27
Diploscapter sp.	2	2.53
Miscellaneous	4	5.07
	Total	126.56

Sample 4. Improved tussock grassland
Plant rhizosphere sampled: Hypochaeris radicata.

Species	%	No. per ml soil
Paratylenchus projectus	21	14.63
Cephalenchus tahus	4	2.79
Tylenchus species 2.	9	6.27
Aphelenchus avenae	2	1.39
Aphelenchus bicaudatus	4	2.79
Pratylenchus penetrans	4	2.79
Tylenchus maiakus	1	0.70
Dorylaimellus sp.	8	5.57
Tylencholaimellus sp.	4	2.79
Tylencholaimus sp.	5	3.48
Nygolaimus (Paravulvus) sp.	3	2.09
Aporcelaimus species 1.	1	0.70
Aporcelaimus species 2.	9	6.27
Dorylaimus species 3.	2	1.39
Wilsonema otophorum	3	2.09
Acrobeloides sp.	2	1.39
Cervidellis species 2.	4	2.79
Mesorhabditis species 2.	2	1.39
Cephalobus sp.	2	1.39
Rhabditis sp.	5	3.48
Genus et sp. indent.	2	1.39
Miscellaneous	3	2.09
	Total	69.66

APPENDIX IV continued

A. Improved tussock grassland

Sample 5.

Plant rhizosphere sampled: Agrostis tenuis.

Species	%	No. per ml soil
Paratylenchus projectus	13	11.26
Tylenchus maiakus	2	1.73
Aphelenchoides bicaudatus	4	3.46
Tylenchus rikus	5	4.33
Aphelenchoides sp.	2	1.73
Tylenchus species 2.	5	4.33
Aphelenchus avenae	6	5.19
Helicotylenchus sp.	1	1.73
Pratylenchus penetrans	4	3.46
Aporcelaimus species 1.	4	3.46
Dorylaimellus sp.	3	2.59
Tylencholaimellus sp.	3	2.59
Tylencholaimus sp.	2	1.73
Nygolaimus (Paravulvus)sp.	4	3.46
Acrobeloides sp.	7	6.05
Eucephalobus sp.	7	6.05
Chiloplacus sp.	8	6.91
Wilsonema otophorum	9	7.79
Monhystera sp.	5	4.33
Miscellaneous	6	5.19
	Total	87.37

APPENDIX IV continued.

A. Improved tussock grassland.

Sample 6.

Plant rhizosphere sampled: Trifolium pratense.

Species	%	No. per ml soil
Paratylenchus projectus	38	38.63
Aglenchus neozelandicus	9	9.30
Tylenchus maiakus	1	1.02
Tylenchus rikus	6	6.10
Pratylenchus penetrans	1	1.02
Ditylenchus species 1.	3	3.05
Aphelenchus avenae	2	2.03
Dorylaimellus sp.	1	1.02
Aporcelaimus species 1.	3	3.05
Aporcelaimus species	5	5.23
Acrobeloides sp.	4	4.07
Cervidellis species 1.	7	7.12
Eucephalobus sp.	5	5.23
Wilsonema otophorum	6	6.10
Anaplectus granulosis	2	2.03
Prismatolaimus sp.	2	2.03
Miscellaneous	5	5.23
	Total	102.26

APPENDIX IV continued

A. Improved tussock grassland.

Sample 7.

Plant rhizosphere sampled: *Trifolium pratense*.

Species	%	No. per ml soil
Paratylenchus projectus	21	28.05
Aglenchus neozelandicus	38	50.69
Aphelenchoides bicaudatus	2	2.66
Dorylaimellus sp.	10	13.41
Aporcelaimus species 1.	4	5.36
Aporcelaimellus paraamylovorus	4	5.36
Dorylaimus sp.	1	1.33
Tylencholaimellus sp.	2	2.66
Nygolaimus (*Paravulvus*) sp.	2	2.66
Eucephalobus sp.	2	2.66
Wilsonema otophorum	9	12.07
Genus et sp. indent.	2	2.66
Miscellaneous	3	4.02
	Total	133.59

APPENDIX IV continued

A. Improved tussock grassland.
Sample 8.
Plant rhizosphere sampled: *Festuca novae-zelandiae*.

Species	%	No. per ml soil.
Paratylenchus projectus	11	6.99
Aglenchus neozelandicus	17	10.81
Tylenchus maiakus	11	6.99
Tylenchus species 2.	4	2.54
Ditylenchus species 1.	7	4.45
Dorylaimellus sp.	17	10.81
Aporcelaimus species 1.	3	1.91
Aporcelaimus species 3.	6	3.82
Aporcelaimellus paraamylovorus	2	1.27
Dorylaim indent.	2	1.27
Tylencholaimellus sp.	4	2.54
Plectus sp. (*P. parietinus* type)	1	0.64
Wilsonema otophorum	2	1.27
Plectus sp. (*P. armatus* type)	1	0.64
Chiloplacus sp.	1	0.64
Acrobeloides sp.	1	0.64
Cervidellis species 1.	1	0.64
Prismatolaimus sp.	2	1.27
Genus et sp. indent.	4	2.54
Miscellaneous	3	1.91
	Total	63.59

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 1.
Plant rhizosphere sampled: Festuca novae-zelandiae.

Species	%	No. per ml soil.
Paratylenchus projectus	7	1.84
Aglenchus neozelandicus	9	2.36
Cricenomoides sp.	8	2.10
Cephalenchus tahus	1	0.26
Tylenchus rikus	20	5.25
Aphelenchus avenae	1	0.26
Aporcelaimus species 1.	2	0.52
Dorylaimus species 2.	3	0.79
Eudorylaimus species 2.	3	0.79
Mylonchulus sp.	4	1.05
Diphtherophera sp.	2	0.54
Acrobeloides sp.	13	3.41
Cephalobus sp.	4	1.05
Rhabditis species 2.	15	3.94
Plectus sp. (P. parietinus type)	2	0.52
Wilsonema otophorum	1	0.26
Rhabdolaimus sp.	4	1.05
Teratocephalus sp.	2	0.52
	Total	26.51

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 2.
Plant rhizosphere sampled:
Agrostis tenuis.

Species	%	No. per ml soil.
Paratylenchus projectus	11	5.95
Aglenchus neozelandicus	1	0.54
Cricenomoides sp.	1	0.54
Tylenchus maiakus	9	4.87
Tylenchus rikus	18	9.74
Tylenchus species 3.	8	4.33
Aphelenchus avenae	4	2.16
Dorylaimus species 2.	13	7.02
Dorylaim species indent.	1	0.54
Tylencholaimus sp.	2	1.08
Mononchus propapillatus	2	1.08
Nygolaimus (*Paravulvus*) sp.	1	0.54
Acrobeloides sp.	3	1.62
Rhabditis species 2.	10	5.39
Cephalobus sp.	7	3.80
Plectus (*P. parietinus* type)	3	1.62
Wilsonema otophorum	1	0.54
Miscellaneous	5	2.71
	Total	54.07

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 3.
Plant rhizosphere sampled:
Whalenbergia albormarginata

Species	%	No. per ml soil
Paratylenchus projectus	4	1.97
Cricenomoides sp.	1	0.49
Cephalenchus tahus	8	3.95
Helicotylenchus sp.	19	9.37
Tylenchus rikus	11	5.43
Tylenchus species 3.	3	1.48
Aphelenchus avenae.	1	0.49
Aporcelaimus species 3.	10	4.93
Aporcelaimus species 1.	3	1.48
Aporcelaimellus paraamylovorus	3	1.48
Tylencholaimus sp.	4	1.97
Dorylaimellus sp.	4	1.97
Nygolaimus (*Paravulvus*) sp.	5	2.47
Mesorhabditis species 3.	2	0.99
Plectus sp. (*P. armatus* type)	4	1.97
Bastiana sp.	3	1.48
Rhabdolaimus sp.	6	2.96
Genus et sp. indent.	8	3.95
Miscellaneous	1	0.49
	Total	49.32

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 4.
Plant rhizosphere sampled:
Hypochaeris radicata.

Species	%	No. per ml soil.
Paratylenchus projectus	6	3.52
Aglenchus neozelandicus	4	2.35
Cricenomoides sp.	7	4.11
Tylenchus maiakus	10	5.86
Tylenchus rikus	16	9.39
Nothotylenchus sp.	1	0.59
Aphelenchus avenae	3	1.76
Aporcelaimus species 1.	3	1.76
Alaimus sp.	2	1.17
Tylencholaimus sp.	4	2.35
Tylencholaimellus sp.	2	1.17
Eudorylaimus species 2.	3	1.76
Nygolaimus (Paravulvus) sp.	4	2.35
Cephalobus sp.	3	1.76
Mesorhabditis species 3.	6	3.52
Acrobeloides sp.	13	7.63
Plectus sp. (P. armatus type)	6	3.52
Wilsonema otophorum	3	1.76
Prismatolaimus sp.	2	1.17
Miscellaneous	2	1.17
	Total	58.67

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 5.
Plant rhizosphere sampled:
Raoulia subsericea.

Species	%	No. per ml soil.
Paratylenchus projectus	6	3.41
Criconemoides sp.	22	12.51
Tylenchus rikus	10	5.68
Tylenchus species 3.	3	2.44
Aphelenchoides bicaudatus	3	1.71
Aporcelaimus species 1.	6	3.41
Aporcelaimellus paraamylovorus	5	2.84
Dorylaimellus sp.	9	5.12
Mononchus propapillatus	4	2.24
Mononchus sp.	1	0.57
Dorylaimus species 2.	4	2.24
Nygolaimus (Paravulvus) sp.	6	3.41
Dorylaimus species 1.	4	2.24
Rhabditis species 3.	8	4.55
Monhystera sp.	4	2.24
Prismatolaimus sp.	1	0.57
Miscellaneous	4	2.24
		Total 57.42

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 6.
Plant rhizosphere sampled:
Agrostis tenuis.

Species	%	No. per ml soil.
Paratylenchus projectus	10	2.73
Aglenchus neozelandicus	4	1.09
Cricenomoides sp.	6	1.63
Tylenchus maiakus	12	3.26
Tylenchus species 3.	8	2.18
Aphelenchoides bicaudatus	5	1.36
Dorylaimellus sp.	4	1.09
Tylencholaimus sp.	8	2.18
Tylencholaimellus sp.	2	0.54
Tylencholaimellus montanus	3	0.82
Aporcelaimus species 1.	2	0.54
Eudorylaimus species 2.	1	0.27
Mononchus sp.	5	1.36
Eucephalobus sp.	4	1.09
Cephalobus sp.	1	0.27
Diplogaster sp.	2	0.54
Plectus sp. (P. armatus type)	4	1.09
Rhabdolaimus sp.	14	4.90
Genus et sp. indent.	3	0.82
Miscellaneous	2	0.54
	Total	28.30

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 7.
Plant rhizosphere sampled:
Hypochaeris radicata.

Species	%	No. per ml soil.
Paratylenchus projectus	10	8.13
Aglenchus neozelandicus	4	3.25
Cricenomoides sp.	6	4.88
Tylenchus maiakus	6	4.88
Tylenchus rikus	10	8.13
Tylenchus species	3	2.44
Aphelenchus avenae	6	4.88
Tylencholaimus sp.	8	6.51
Tylencholaimellus montanus	2	1.63
Dorylaimellus sp.	10	8.13
Diptherophera sp.	2	1.63
Aporcelaimellus paraamylovorus	4	3.25
Dorylaim genus et sp. indent.	2	1.63
Acrobeloides sp.	10	8.13
Bastiana sp.	1	0.81
Wilsonema otophorum	3	2.44
Plectus sp. (P. armatus type)	3	2.44
Plectus sp. (P. inquirendus type)	2	1.63
Genus et sp. indent.	5	4.07
Miscellaneous	3	2.44
	Total	81.33

APPENDIX IV. continued.

B. Unimproved tussock grassland.
Sample 8.
Plant rhizosphere sampled:
Festuca novae-zelandiae

Species	%	No. per ml soil.
Aglenchus neozelandicus	26	9.18
Cephalenchus tahus	10	3.53
Tylenchus rikus	4	1.41
Aphelenchus avenae	9	3.18
Nothotylenchus sp.	1	0.35
Dorylaimellus sp.	4	1.41
Aporcelaimus species 1.	2	0.71
Aporcelaimellus paraamylovorus	2	0.71
Tylencholaimus sp.	8	2.82
Eudorylaimus species 2.	8	2.82
Mononchus propapillatus	3	1.06
Rhabditis species 2.	11	3.88
Anaplectus granulosis	4	1.41
Wilsonema otophorum	6	2.12
Genus et sp. indent.	2	0.71
	Total	35.30

Appendix V. Determination of transformation exponents for summer and winter samples using Taylor's power law.

Category	Season	Regression of variance on mean		Significance of regression by 't' test	Exponent
		Constand	Gradient		
Total nematodes	Summer	0.2480	1.0697	1.4418	0.46517
	Winter	2.4789	-0.0963	-0.1112	1.04813
Higher plant feeders	Summer	-0.6602	1.7286	2.2047	0.13571
	Winter	0.9174	0.7545	0.5543	0.62275
Filamentous feeders	Summer	-2.6417	3.9279	15.0902**	-0.96396
	Winter	-0.2712	1.7248	3.4388	0.13761
Bacterial feeders	Summer	3.3685	-1.9614	-0.6226	1.98071
	Winter	0.3173	0.8504	0.7658	0.57478
Miscellaneous feeders	Summer	-0.1312	1.6793	2.4808	0.16031
	Winter	0.6802	0.1965	0.2801	0.90173
Predators	Summer	0.0790	1.6618	1.4107	0.16908
	Winter	0.05548	1.54242	1.8605	0.22879

$t_{(2\ D.F.)}$

0.05 = 4.303 *

0.01 = 9.925 **

Appendix VI. Determination of transformation exponents for samples from ascending and descending temperature series.

Category	Season	Regression of variance on mean		Significance of regression by 't' test	Exponent
		Constant	Gradient		
Total nematodes	Ascending	-2.1010	2.2374	16.5174**	-0.11871
	Descending	0.6397	0.9196	0.8357	0.54020
Higher plant feeders	Ascending	1.6417	0.3674	0.2933	0.81627
	Descending	-4.4138	4.0374	2.2793	-0.01870
Filamentous feeders	Ascending	-0.3743	1.8185	3.7702	0.09077
	Descending	-0.1481	1.3353	0.6542	0.33233
Bacterial feeders	Ascending	3.4629	-2.0514	-0.9046	2.02568
	Descending	0.0782	1.0761	0.9533	0.46195
Miscellaneous feeders	Ascending	-1.7140	3.4829	1.3074	-0.74143
	Descending	0.9211	-0.4091	-0.8976	1.20455
Predators	Ascending	0.2911	0.3611	1.1704	0.81943
	Descending	-0.13098	0.0627	0.5592	0.68639

$t_{(2\ D.F.)}$

0.05 = 4.303 *

0.01 = 9.925 **

APPENDIX VII. Improvement of the germination percentage of *F. novae-zelandiae* seed by cold treatment.

Method

All seed was surface sterilised in a 4:1 solution of 0. 1% mercuric chloride and 95% alcohol for 10 minutes, rinsed in sterile distilled water, and dried between sterile filter pads. Two hundred seeds for each of the following treatments were germinated on moist blotters at 20°C under artificial lights (day length, 16 hours), and the germination percentage recorded after 10 days and 25 days:

i) No post surface sterilisation treatment;
ii) Incubation at 4°C for 1 week;
iii) Incubation at 4°C for 2 weeks;
iv) Incubation at 4°C for 3 weeks;
v) Incubation at 4°C for 4 weeks;
vi) Incubation at 4°C for 5 weeks;
vii) Incubation at 4°C for 6 weeks.

Results

Period of cold treatment (weeks)	Germination % 10 days (interim)	25 days (final).
0	13	36
1	31	51
2	19	42
3	30	73
4	41	60
5	36	70
6	35	59